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Analysis of the Contribution of Charge Transport in Iodine-125 induced DNA Damage

Thabisile Ndlebe¹, Igor Panyutin^{1,2}, and Ronald Neumann¹

¹Radiology and Imaging Sciences, Clinical Center, NIH, Bethesda, MD 20892

Abstract

Auger electron emitters, like iodine-125, are the radionuclides of choice for gene-targeted radiotherapy. The highly localized damage they induced in DNA is produced by three mechanisms: direct damage by the emitted Auger electrons, indirect damage by diffusible free radicals produced by Auger electrons travelling in water, and charge neutralization of the residual, highly positively charged, tellurium daughter atom by stripping electrons from covalent bonds of neighboring residues. The purpose of our work was to determine whether these mechanisms proceed through an intermediate energy transfer step along DNA. It was proposed that this intermediate step proceeds through the charge transport mechanism in DNA. Conventional charge transport has been described as either a hopping mechanism initiated by charge injection into DNA and propagated by charge migration along the DNA, or a tunneling mechanism in which charge moves directly from a donor to an acceptor within DNA. Well-known barriers for the hopping mechanism were used to probe the role of charge transport in ¹²⁵I induced DNA damage. We studied their effect on the distribution of DNA breaks produced by the decay of iodine-125 in samples frozen at –80°C. We found that these barriers had no measurable effect on the iodine-125 breaks distribution.

INTRODUCTION

For the last several decades the therapeutic potential of radionuclides as sequence-specific DNA damaging agents has been an area of interest (1-3). In particular, studies using the ¹²⁵I radionuclide have shown that it induces a distinct pattern of damage in DNA (4,5). Most of the ¹²⁵I breaks occur proximal to the decay site (initiation site). The mechanism of ¹²⁵I induced DNA damage reportedly consists of three components (5,6). The first component is the direct damage by the emitted Auger electrons, the second component is the indirect damage by diffusible free radicals produced by Auger electrons' reaction with water, and the third component is charge neutralization of the highly positively charged tellurium daughter atom by the stripping of electrons from covalent bonds of neighboring residues. The charge neutralization component was proposed to be dominant within 4–5 bases from the decay site; while direct damage by the emitted Auger electrons was proposed to be dominant beyond 8–9 bases from the decay site (6).

Although the main components of the mechanism of DNA damage for ¹²⁵I have been elucidated, some studies suggested a minor role for charge transport in ¹²⁵I induced DNA damage (7-9). In earlier experiments with plasmid DNA, unexpected long distant DNA damage was observed (100 base pairs away). Several explanations for this observation were proposed

²Corresponding Author: Igor Panyutin, National Institutes of Health, Radiology and Imaging Sciences, Clinical Center, Building 10, Rm. 4D45, Bethesda, MD 20892, ipanyuting@mail.nih.gov
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(7,9,10). One proposal was that this distant DNA damage was the result of charge transport along DNA. Independently, a modeling study attempted to incorporate this charge transport mechanism into the model of ^{125}I induced DNA damage (8). The results of this study suggested that charge transport could possibly play a role in the charge neutralization of the tellurium daughter atom.

Charge transport is a well-established mechanism of long distance damage in DNA (11-16). Numerous studies have shown that charge (either radical cations or anions) within DNA can migrate over large distances, and subsequently lead to damage within the DNA (17-19). It was also shown that charge transport plays a role in the distribution of DNA damage produced by ionizing radiation (14,16). The two main mechanisms of charge transport that have been described are the tunneling and hopping mechanisms. The tunneling or superexchange mechanism is the transport of the charge directly from the donor to the acceptor, while the hopping mechanism describes the charge transport from the donor through discrete steps along the DNA (19). For the purposes of this study, charge migration describes a series of consecutive single step charge transfer events that occur over a distance in DNA. They continue along the DNA until they are either quenched by the environment (water, oxygen, etc) or react with a base (trapped). This process results in oxidative damage at different sites along DNA.

Charge transport could either proceed through hole transport, where a radical cation is transported along the DNA, or through excess electron transfer/transport (EET), where a radical anion is transported along the DNA (13). Over the years, different reviews of charge transport have described hole transport (radical cation migration) and excess electron transport (radical anion migration) as proceeding through either hopping or tunneling or a combination of both hopping and tunneling mechanisms (13,19).

Hole transport is the more widely studied type of charge transport (17-19). Studies of hole transport in DNA have shown that the lower oxidation potential of guanine makes it the most efficient radical cation trap among all normal DNA bases. A stronger trapping effect was observed when two (GG) or more consecutive guanine bases were present (20). This GG step selectivity implied that hole transport was both a distance and sequence-dependent mechanism as it showed that the amount of damage at a proximal GG step (close to charge injection site) was more than the amount at distal GG steps depending on which DNA sequence was used.

Physical barriers (such as DNA gaps (20) or mismatches (21)) and chemical barriers (such as 8-oxo-7,8-dihydroguanine (8-oxo-G) (22)) have been used to characterize hole transport in DNA. Charge transport is extremely sensitive to DNA stacking interruptions and DNA dynamics. Structural studies of nicked, gapped and mismatched DNA demonstrated that the major species are close to canonical B form DNA albeit with more local flexibility (23,24). A previous study reported that although gaps, mismatches and bulges inhibited hole transport, they were unable to completely inhibit it (20). For mismatched DNA, studies showed that their effect was dependent on their exact location within the DNA sequence (25). It was also reported that the type of mismatch (CA or GT or CC, etc.) determines the mismatch's ability to inhibit hole transport in DNA (21). Using biochemical methods, these authors showed that CA mismatches had one of the most hindering effects on hole transport. Structural studies of 8-oxo-G containing samples maintained B conformation in DNA (26). Kanvah and Schuster reported that depending on the sequence used, most hole transport in DNA is inhibited by 8-oxo-G rather than GG steps (20,22). This was attributed to 8-oxo-G having a lower oxidation potential than guanine.

Excess electron transport (EET) involves the generation of a radical anion due to electron injection into DNA. The radical anion migrates along DNA and induces preferential damage at modified bases (27). EET has often been characterized by using electron acceptors such as

thymine dimers and 5-bromo-2'-deoxyuridine (BrdU) to serve as chemical probes for radical anions within DNA (28,29). Upon reaction with a radical anion, BrdU undergoes one electron reduction that subsequently leads to DNA strand cleavage (27,28,30). At low temperatures (below 80K), EET by the tunneling mechanism has been observed whereas EET by the hopping mechanism has been observed at higher temperatures (31-33). Due to their lower reduction potentials, cytosine and thymine bases are expected to be most easily reduced (13).

However, in order to fully determine whether charge transport could potentially play a role in ^{125}I decay induced DNA damage, it is important to understand the details of ^{125}I decay. Iodine-125 decay proceeds through two cascades and a charge neutralization event. The first cascade proceeds as a result of the electron capture of an inner shell orbital electron by the ^{125}I nucleus leading to its decay into a metastable tellurium daughter atom. The binding energy of the captured electron is then released by the emission of Auger electrons, x-rays and other electrons. Then the second cascade proceeds through the release of internal conversion electrons and x-rays. As a result, it is quite possible that the emission of all these electrons could lead to events that lead to EET. Also, the metastable tellurium daughter atom extracts electrons from its parent molecule and neighboring molecules in order to neutralize its highly positive charge. During this process, it is also possible that an electron could be withdrawn from a neighboring nucleobase leading to the formation of a radical cation and resulting in hole transport through the DNA. Interest in determining the exact extent of these implications of the involvement of charge transport in ^{125}I induced DNA damage led to this study.

Our objective was to determine whether charge transport is a component of ^{125}I induced DNA damage by using known charge transport barriers to probe the presence of charge transport. These barriers were either chemical charge transport barriers (such as the chemically modified bases, 8-oxo-G and BrdU) or physical charge transport barriers (such as gapped and mismatched DNA). Modified bases report the presence of charge transport by reacting with either the radical cation or the radical anion, respectively. This is observed as more visible damage at these bases compared to the background. The barriers were incorporated into short oligomer sequences. These short oligomers were then annealed to DNA hairpin templates (HT). Then, depending on the sample, they were ligated to form full hairpin sequences (Table 1) which were used to analyze the effects of the barriers on charge transport in ^{125}I DNA damage. DNA hairpins were used for their general convenience (easier handling and interpretation of results). Variations in the breaks distribution patterns of the samples containing these barriers compared to controls (without barriers) were used to show any evidence for charge transport. The results of our study show that charge transport is probably not a key component of ^{125}I decay- induced DNA damage.

MATERIALS AND METHODS

DNA Sample Preparation

DNA samples were synthesized on an ABI 394 Synthesizer (Applied Biosystems, Foster City, CA). Table 1 contains the full hairpin DNA sequences. Modified oligonucleotides containing 5'-Dimethoxytrityl-N2-isobutyryl-8-oxo-deoxyGuanosine-3'-[(2-cyanoethyl)-(N, N-diisopropyl)] (8-oxo-G) or 5'-Dimethoxytrityl-5-bromo-2'-deoxyUridine, 3'-[(2-cyanoethyl)-(N, N-diisopropyl)] (BrdU) phosphoramidites were synthesized as specified by the manufacturer (Glen Research, Sterling VA). All samples were further purified with 12% denaturing polyacrylamide gel electrophoresis (PAGE) (National Diagnostics, Atlanta, GA), and gel filtration through MicroSpin G-25 or MicroSpin G-50 columns (GE Healthcare, Piscataway, NJ) depending on their size.

Short DNA Oligomer Radiolabeling

All short oligomers (21-mer or 22-mers) were first 5' phosphorylated with T4 polynucleotide kinase (Fermentas, Glen Burnie, MD) in reaction buffer supplemented with 1 mM ATP prior to annealing or ligation. The short oligomers were then radiolabeled with ^{32}P on their 3' end with Terminal deoxynucleotidyl transferase (Fermentas). Samples were also purified with MicroSpin G-25 columns to remove unincorporated radionucleotides.

DNA Template Radiolabeling

Samples of HT were labeled with ^{125}I by incorporating ^{125}I -dCTP (Perkin Elmer, Waltham, VA) to the 3' end by the Klenow Fragment of DNA polymerase I (Fermentas). In addition to ^{125}I -dCTP, the 3' end of HTs was capped by incorporation of either a dGTP or TTP depending on the HT sequence (Table 1). The control samples were obtained by ligation between unlabeled hairpin templates (without ^{125}I) and 3' ^{32}P -labeled short oligomers. These control HT samples also underwent Klenow Fragment template extension except unlabeled dCTP was used instead of ^{125}I -dCTP.

DNA Annealing and Ligation Conditions

Samples were annealed by cooling from 90°C to room temperature for 1 hr in 10mM Tris-HCl, pH 7.4 and 1 mM EDTA (TE) buffer with 50 mM NaCl. Previous studies have shown that Tris-Cl buffer is a sufficient free radical scavenger at millimolar concentrations and low temperatures (4,34). The annealed samples (nicked and gapped samples) were then stored at -20°C until all the samples were ready for incubation at -80°C. The complete full-length hairpins were formed by ligation with T4 DNA Ligase (Fermentas) overnight at 16°C. After ligation with T4 DNA ligase, these samples were gel purified by PAGE as described above. Both annealed and ligated samples were incubated for 2 weeks at -80°C in order to exclude all secondary reactions such as water radiolysis or free radical damage. Then they were analyzed for DNA breaks after 2 weeks using 12% denaturing PAGE. DNA bands were quantified using a BAS-2500 Bioimager (FUJI Medical Systems USA, Stamford, CT).

Accumulation of DNA Damage at -80°C

The half-life of the ^{125}I radionuclide is approximately 60 days. For the accumulation of decay, samples were flash frozen in liquid nitrogen then kept at -80°C (in Tris Buffer) for two weeks, as previously described (35). After incubation at 80°C, the samples were allowed to equilibrate to room temperature. Half the samples were stored at -20°C while the rest were piperidine treated, as described below. After piperidine treatment, all the samples were mixed with stop solution and their counts were adjusted to deliver equivalent counts per gel lane. Previous work has shown that there is little difference between either freezing the samples (at -70°C) without DMSO or storing them in the presence of DMSO (a well known free-radical scavenger)(4, 36).

Piperidine Treatment

Samples were piperidine treated by heating at 90°C in the presence of piperidine for 30 minutes, then they were lyophilized. The purpose of the piperidine treatment was to expose base labile lesions.

BrdU Excision Mix

The BrdU Excision Mix (Epicenter Biotechnologies, Madison, WI) contained both Uracil DNA glycosylase, which removes a uracil base to form an abasic site, and Endonuclease IV, which cleaves the phosphodiester bond at the abasic site leaving a strand break at the site. The treatment was conducted according to the manufacturer's protocol.

DNA Strand Break Analysis

DNA strand breaks were quantified using a BAS-2500 Bioimager and ImageGauge software. An intensity profile was generated for each lane in the gel image. The individual band intensities were obtained from the heights of each peak. The peak heights roughly correlated with the DNA damage at a particular base. The frequency of breaks were defined as a fraction of the peak height of the band intensity for each base over the peak height for the band intensity that corresponded with the total uncut DNA for that lane, respectively.

Due to the magnitude and proximity of the uncut DNA peaks to the ^{125}I induced DNA breaks (Fig. 1), peak deconvolution was performed for both *G* and *N* samples. Uncut DNA peaks overlapped into the peak intensities of the 3 most proximal bases. In order to compensate for this overlap, it was necessary to fit baselines for these peak intensities. The peak heights obtained for the individual band intensities were based on these fitted baselines.

The background correction for the modified base, 8-oxo-G, was calculated as follows. First, loading differences between the sample lanes for *8X* and *8X.i* were compensated by first selecting individual intensity bands at the same position in both sample lanes. Then a ratio was obtained for these two sample lanes and used to adjust all the values in one sample to determine the approximate values under equal loading conditions. The background correction was then derived by deducting the band peak height at the 8-oxo-G position in *8X* (without ^{125}I) from the band peak height at the 8-oxo-G position in *8X.i* (with ^{125}I). Background corrections were calculated in the same manner for BrdU samples, *BU* (without ^{125}I) and *BU.i* (with ^{125}I).

As previously described (4), the probability of a strand break in the hairpin was calculated based on the assumption that the probability of ^{125}I induced breaks is 1. The recursive formula used was:

$$p_i = F_i / [(1 - p_{(i-1)})(1 - p_{(i-2)}) \dots (1 - p_1)] \quad (1)$$

where F_i is the frequency of breaks at the nucleotide in *i*-th position. The formula (p_i) describes the probability of breaks at one base in relation to the probability of breaks for its 3' neighboring bases.

The probability of breaks due to piperidine treatment (to show the contribution of base labile lesions) was determined by first obtaining the frequency of breaks values for (+) piperidine (treated with piperidine) and (−) piperidine (untreated) samples, then subtracting the frequency of breaks values for the (−) piperidine samples from those of the (+) piperidine samples. The probability of breaks due to piperidine were then calculated from the above mentioned recursive formula using the values obtained from this subtraction.

The estimated experimental error for the breaks frequency measurements was in the range of 10%, based on three independent analyses. More accurate error limits were challenging to obtain because the results were mainly based on the amount of ^{125}I incorporated in each strand, and each independent iodine-125 incorporation experiment results in different yields of ^{125}I incorporation into the hairpin template strand.

RESULTS

Effect of gapped and mismatched DNA on charge transport in iodine-125 induced DNA Damage

DNA samples containing either a gap (missing nucleotide) or mismatched base pairs as barriers to charge transport were used to study the possible role of this process in ^{125}I induced DNA

damage (Fig. 1). A nicked DNA sample (*N*) containing a nick one base away from ^{125}I -dC served as the control for the gapped DNA sample (*G*) (missing a nucleotide one base away from ^{125}I -dC). The presence of the nick in DNA resulting from a missing phosphodiester bond does not perturb the stacking interactions between DNA bases, and therefore should not impede charge transport. The samples were analyzed by PAGE after two weeks of incubation at -80°C (Fig. 1). They showed the characteristic pattern of ^{125}I -induced DNA damage with almost 90% of the breaks found within 10 base pairs from the initiation site, and a distance dependent decrease in breaks from this site (Fig. 1, Lanes 1 & 2, [brackets]).

The intensities of the uncut DNA bands for *N* and *G* were measured and normalized as described in the Materials and Methods. The resulting values are shown in Fig. 2A. Due to the large size of the uncut DNA peaks, and their proximity to the ^{125}I induced DNA breaks, peak deconvolution was necessary for the *N* and *G* samples. The analysis for the *N* and *G* samples was performed in relation to the position away from the uncut DNA band (Fig. 2A & 2B), where the first cleavage band beside the uncut DNA (the 5' end of the short oligonucleotide) was labeled position -1 . Although peak deconvolution was performed for these samples, positions -1 to -3 still appeared to be affected by peak overlapping. The frequency of breaks did not vary much beyond position -3 from uncut DNA.

The probability of breaks, which account for the possibility of multiple breaks occurring within a single molecule, were calculated from the frequencies of DNA breaks using the recursive formula described in the Materials and Methods. The results showed a very small difference between the probability of breaks for *G* versus the control *N* (Fig. 2B). This suggests that the gap barrier has very little effect on the probability of breaks distribution for ^{125}I -induced DNA damage.

We then studied the effect of mismatches in the DNA hairpins (Table 1) on the distribution of ^{125}I -induced breaks. Mismatched DNA samples either contained one CA mismatch two bases away from ^{125}I -dC (*M1*) or two CA mismatches four bases away from ^{125}I -dC (*M2*), respectively. The control samples did not contain mismatches and were either not labeled with ^{125}I (*MC*) or contained ^{125}I (*MC.i*). Unusual degradation of control oligonucleotides (Figure 1, Lane 3) was likely due to random radiolysis and did not significantly affect the overall accuracy of this conclusion.

Figure 1 shows the characteristic ^{125}I DNA damage pattern in ^{125}I containing samples, *MC.i*, *M1* and *M2* (Fig. 1, Lanes 4–6). The corresponding probabilities of frank breaks are shown in Figure 2C. No difference was observed between the probability of breaks of *MC.i*, *M1* or *M2* (Fig. 2C). This suggests that there was no apparent effect of mismatched DNA on ^{125}I -induced DNA breaks.

Overall, when using a gap or mismatches as charge transport barriers in DNA, we found no appreciable change in the distribution of ^{125}I induced breaks in DNA.

Effect of 8-oxo-G on charge transport in iodine-125 induced DNA Damage

We then assessed the effect of charge transport in ^{125}I induced DNA damage using a modified base, 8-oxo-7,8-dihydroguanine (8-oxo-G). Figure 3 showed the results of the ^{125}I decay on DNA samples, which were either (–) piperidine (Lanes 1–4) or (+) piperidine (Lanes 5–10). An 8-oxo-G was incorporated 4 bases away from the ^{125}I -dCTP in *8X.i*, and at the same position in *8X* (without ^{125}I). All the other control samples were without 8-oxo-G. They either contained ^{125}I (*8C.i*) or not (*8C*). Some nonspecific degradation of the control oligonucleotides was also observed in Figure 3, and was most likely due to random radiolysis. The overall analysis of the breaks distribution showed that the regions of interest (Fig. 3 - in the ^{125}I induced breaks region and the 8-oxo-G position) were not significantly affected by this nonspecific

degradation. The frequency of breaks values were obtained from the intensity profiles generated from these gel images (Fig. 3), and the probability of breaks was calculated from the frequency of breaks values (Fig. 4).

First, we determined whether the presence of 8-oxo-G affected the distribution of direct ^{125}I induced DNA strand breaks. The probability of breaks for these (–) piperidine samples showed little effect on the overall distribution of frank breaks due to ^{125}I in the presence of 8-oxo-G (Fig. 4A). The only localized effect observed was a very slight decrease at the thymine base proximal to the ^{125}I in sample **8X.i** when compared to sample **8C.i**. This suggests that 8-oxo-G had little apparent localized effect on the direct ^{125}I induced DNA strand breaks.

Next, we determined whether charge transport was involved in the ^{125}I induced DNA damage by performing a closer analysis of the 8-oxo-G probe itself. Generally, unmodified 8-oxo-G reacts slowly with piperidine to form DNA strand breaks at this position. Once it undergoes oxidation by a radical cation, it generates additional oxidative products that are even more piperidine labile (22). Therefore, an observation of any increase in the piperidine labile products at the 8-oxo-G base after ^{125}I decay (compared to the 8xoG control) and piperidine treatment ((+) piperidine) would suggest that oxidation occurred at this position and would provide evidence for charge transport upon ^{125}I decay in DNA. The results of the breaks analysis in (+) piperidine samples are presented in Figure 4B. An increase in the probability of breaks was observed at the 8-oxo-G position of **8X.i**. However, to accurately make this assessment, a background correction for the unoxidized 8-oxoG (found in **8X**) was required. The background correction was performed as described in the Materials and Methods. The frequency of breaks obtained from the intensity of the piperidine-induced bands in unoxidized, non- ^{125}I -labeled sample (**8X**) were subtracted from those in ^{125}I labeled sample (**8X.i**). The background corrected results showed that there was no real difference between damage in **8X.i** compared to **8C.i** (control with iodine and no 8-oxo-G)(see Fig. 4C). These results show that 8-oxo-G did not undergo further oxidative damage after the decay of ^{125}I . This suggests that charge transport (or rather radical cation migration along DNA) does not play a significant role in ^{125}I DNA damage.

In spite of the absence of a strong localized effect of 8-oxo-G, it could possibly have a global effect on the distribution of the DNA breaks after piperidine treatment. The probability of breaks due to piperidine treatment (showing the contribution of base labile lesions) were calculated by subtracting the frequency of breaks for (–) piperidine samples from the (+) piperidine samples (Fig. 4D). The overall pattern of the probability of breaks due to piperidine treatment alone was similar for **8X.i** and **8C.i** (Fig. 4D). The presence of the 8-oxo-G within the DNA strands had no global effect on the probability of piperidine labile breaks. This confirms that under these conditions, charge transport due to radical cation migration does not play a role in ^{125}I induced DNA damage.

Effect of BrdU on charge transport in iodine-125 induced DNA Damage

To determine whether the alternative mode of charge transport, excess electron transfer/transport (EET), played a role in ^{125}I induced DNA damage, BrdU was incorporated into ^{125}I labeled DNA. It was incorporated 3 bases away from the ^{125}I -dC position. The control samples were either without ^{125}I -dC (**BC and BU**) or with ^{125}I -dC (**BC.i**). All the control samples, besides **BU**, were without BrdU. The experimental sample, **BU.i**, contained both ^{125}I -dC and BrdU. The samples were treated with BrdU excision mix and analyzed by PAGE analysis. Both **BU.i** and **BC.i** had the same characteristic ^{125}I pattern of DNA strand cleavage (Fig. 5, Lanes 5 and 6, respectively) with the breaks decreasing in a distance dependent manner. Prior to background correction, their probability of breaks analysis showed an increase at the BrdU position for the **BU.i** sample (Fig. 6A). However, once the **BU.i** sample was background corrected a similar breaks distribution was observed at the BrdU position for both

the *BU.i* and the *BC.i* samples. The background corrected probability of breaks analysis for these samples did not show any variation between *BU.i* and *BC.i* (Fig. 6B). This suggested that BrdU did not have a localized or global effect on the probability of breaks. Also, these results show that EET does not affect ^{125}I induced DNA damage.

Taking into account the inherent limitations of the experimental tools and methods used, the overall results suggest that conventional charge transport does not play a significant role in ^{125}I induced DNA damage.

DISCUSSION

The purpose of this work was to determine whether ^{125}I induced DNA damage proceeds in part through an intermediate energy or charge transport step along DNA. Several studies have suggested the presence of an intermediate charge transport step in ^{125}I induced DNA damage. In order to address this, physical (gap and mismatches) and chemical (8-oxo-G and BrdU) barriers to charge transport through DNA were used. Their effects were determined by localized or global changes in the distribution of ^{125}I induced DNA breaks.

Previous studies on ^{125}I decay induced DNA damage proposed conflicting theories on the exact limits of the DNA damaging range of ^{125}I decay in DNA. Lobachevsky and Martin reported that ^{125}I decay induced DNA damage dominates within nine nucleotides from the ^{125}I decay site, and that indirect damage by scavengeable free radicals may dominate beyond this range (5,6,37). Earlier reports also showed that the range of ^{125}I decay damage was dominant between 15 to 20 Å from the decay site and extended up to 70 Å (about 20 base pairs) (37). Studies on plasmid DNA showed “long range” damage beyond these limits (7,37). Linz and Stocklin presented evidence of ^{125}I decay induced DNA damage hundreds of base pairs away from the ^{125}I -dC site (7); while Winters *et. al.* suggested long distant damage beyond twenty four base pairs (9). These latter studies suggested a charge transport component in ^{125}I induced DNA damage. An alternative explanation proposed was that the long distance damage observed in plasmid DNA was due to high-energy Auger electrons released from ^{125}I decay (10).

As mentioned earlier, a more recent model of ^{125}I decay induced DNA damage was generated based on the charge transport theory (8). Earlier work showed that the charge transport rate was of the order of about 10^{-13} s (8,38,39). However, in the ^{125}I decay process, the ^{125}I decays into a metastable state ($^{125\text{m}}\text{Te}$) followed by the emission of 10 Auger electrons in 10^{-15} s, then $^{125\text{m}}\text{Te}$ decays into ^{125}Te followed by the emission of 10 Auger electrons in about 10^{-9} s (40). Therefore, the charge transport rates are comparable to the first step and much faster than the second step. Based on this, the results from this modeling study suggested that charge might not build up on the $^{125\text{m}}\text{Te}$ atom, but be transferred along and damage neighboring DNA bases.

Studying the contribution of charge transport within ^{125}I decay induced DNA damage presents a few inherent challenges. First, the frank breaks produced by the ^{125}I decay mask the most important characteristic features of charge transport such as GG selectivity, distance dependence and the piperidine-labile DNA damage pattern(20). These are all key attributes describing conventional charge transport. In hole transport, charge migrates freely until it reaches a low oxidation potential GG step, and react with it (is trapped at these bases). This results in a GG selective pattern of damage. Distance-dependence is obtained from the ratios of the amounts of damage at proximal (close to initiation site) GG step compared to the distal GG step. This reveals important details about charge transport efficiencies and sequence effects. Variations in the piperidine-labile DNA damage pattern reveal interesting information such as the k_{trap} (charge trapping rate) and the k_{hop} (charge migration rate) (11). Previous studies have shown that $(\text{AGGA})_n$ sequences were more efficient barriers for hole transport

(41). Although (AGGA)_n sequences were used in this study, the characteristic patterns of damage obtained in conventional charge transport were overshadowed by these ¹²⁵I induced frank breaks. In the absence of the wealth of information obtained from the piperidine-labile DNA damage pattern, chemical probes such as 8-oxo-G or BrdU were used to overcome this problem in the current study. Oxidation of 8-oxo-G should be clearly observable upon piperidine treatment despite the ¹²⁵I decay induced DNA breaks. Our results demonstrated this by clearly showing that the intensity of the band that corresponded to 8-oxo-G was more intense than the band intensities that corresponded to the ¹²⁵I frank breaks (Fig. 3). Similar results were obtained for BrdU (Fig. 5).

Second, charge transport mostly results in base labile lesions and not direct strand breaks (11,12). These base labile lesions chemically modify DNA and then require either heat, alkali or enzymatic treatment in order to obtain DNA strand breaks. Frank breaks obtained from charge transport are often due to the generation of free radicals such as H[•] and OH[•] that lead to direct strand scission (12). Lobachevsky and Martin (5) showed that dimethylsulfoxide (DMSO), a hydroxyl radical scavenger, had a small overall effect on the efficiency of ¹²⁵I decay induced DNA breaks, which suggested that these radicals made a small contribution to the breaks produced by ¹²⁵I. Most charge transport mechanisms that lead to any DNA damage are base labile lesions that require piperidine treatment in order for the breaks to be observed. To reveal these base labile lesions, we treated half the samples with piperidine prior to PAGE analysis (data not shown for gap and mismatched DNA).

In certain cases, mismatched DNA has been shown to impede charge transport. Barton and Bhattacharya showed that mismatches that did not involve a guanine (such as AA or CC or CA mismatches) attenuated charge transport more than guanine containing mismatches (such as GG or GT or GA)(21). Also, depending on the identity of the mismatches, contiguous mismatches have been shown to considerably hinder charge transport through DNA. However, mismatched DNA samples in this study did not show any effect on ¹²⁵I induced DNA breaks. There was also no appreciable difference in both the global and localized distribution of ¹²⁵I induced DNA breaks for gapped DNA samples. The results suggest that charge transport was not a significant factor in this system. The physical probes used for our study did not show any evidence for charge transport in ¹²⁵I decay induced DNA damage.

In certain DNA sequences, 8-oxo-G dramatically reduces hole transport in DNA (22). Since purine-rich sequences such as (AGGA)_n enhance hole transport along DNA, an (AGGA)_n sequence was used for our 8-oxo-G experiments. 8-oxo-G is a more efficient hole trap than GG steps along DNA. Therefore, it served as an ideal probe for hole transport. On the other hand, BrdU has mainly been used as an indicator of the presence of electron transport (30). Although 8-oxo-G and BrdU showed preferential cleavage compared to the surrounding bases, this preferential cleavage did not increase above the background under these experimental conditions. Further, the lack of evidence of GG selective damage further argues that hole transport is not apparent in this work. In our experiments, both chemical probes showed an absence of long distance charge transport in ¹²⁵I decay induced DNA damage.

Although this study used 72mer hairpins with 34 base pair stretches, the results obtained for the chemical probes showed that charge transport was not significant proximal to the ¹²⁵I decay site; and it suggests that it was even less likely to occur at more distal locations along the DNA length. Therefore, the distal ¹²⁵I breaks observed earlier may be induced by either diffusible radicals or by higher-energy Auger electrons.

Some consideration of the effect of low temperature is important for this study as all the samples were incubated at 193K (−80°C) for two weeks. Previous studies have shown that DNA is still an effective electron or hole trap at temperatures around 200K (14,15,42). They also showed

that hopping mechanisms still dominate tunneling at this temperature range. Electron spin resonance studies showed that 8-oxo-G is still able to trap holes at 77 K(15). Therefore, 8-oxo-G is expected to still be able to trap holes at more elevated low temperatures near 200K. Another bromine modified DNA base, 5-bromo-6-hydroxy-5, 6-dihydrothymine, was also shown to efficiently trap excess electrons at 77K (43). Based on these studies, we expect that hole and excess electron transport via the hopping mechanism should still be observed at temperatures near 200K (14,15,43), and the chemical probes (8-oxo-G and BrdU) used in this study are appropriate for this temperature range. Although temperature effects cannot be completely ruled out, these previous studies support the assumption that experiments performed at -80°C are still valid for any discussion of charge transport in ^{125}I .

As mentioned earlier, the mechanism of radio-damage due to the ^{125}I decay in DNA is a unique DNA damaging system that proceeds through two electron cascades and charge neutralization. The exact details describing how these key parts interplay and induce damage to DNA has not been completely resolved. However, our results suggest that this DNA damaging system is fully self-contained. That is, all the strand cleavage, base damage, charge recombination and quenching events must proceed much more proximally and efficiently than hole transport through DNA. As a result, hole transport has little or no chance to occur.

In conclusion, our study shows that charge migration along DNA does not play a substantial role in ^{125}I decay induced DNA damage at 80°C . The various tools we used showed the absence of an effect in the presence of ^{125}I within the first five bases from the ^{125}I initiation site. These results suggest that ^{125}I decay induced DNA damage is most probably due to direct ionizations and excitations of the DNA backbone. This leads to the accumulation of single stranded frank breaks, which subsequently form double stranded breaks (44). Although there is little evidence to support this (12), it is possible that the direct ionizations and excitations that occur as a result of ^{125}I decay could result in internal deoxyribosyl hydrogen atom abstraction and form base radicals which subsequently lead to the double stranded breaks observed in ^{125}I decay induced DNA damage. This would explain the steep distance dependence observed for ^{125}I decay induced DNA damage. Based on these results, further inquiries are necessary to further address the quantum chemistry questions that arise from this work.

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Abbreviations used

8-oxo-G	8-oxo-7,8-dihydroguanine
EET	excess electron transfer/transport
GG	two adjacent guanine bases in a DNA strand
HT	hairpin template
K_{hop}	radical anion or cation hopping rate
K_{trap}	Radical anion or cation trapping rate

REFERENCES

1. Panyutin IG, Neumann RD. The potential for gene-targeted radiation therapy of cancers. Trends in biotechnology 2005;23:492–496. [PubMed: 16125814]

2. Kassis AI. Therapeutic radionuclides: biophysical and radiobiologic principles. *Seminars in nuclear medicine* 2008;38:358–366. [PubMed: 18662557]
3. Kassis AI, Adelstein SJ. Radiobiologic principles in radionuclide therapy. *J Nucl Med* 2005;46(Suppl 1):4S–12S. [PubMed: 15653646]
4. Panyutin IG, Neumann RD. Radioprobng of DNA: distribution of DNA breaks produced by decay of ¹²⁵I incorporated into a triplex-forming oligonucleotide correlates with geometry of the triplex. *Nucleic acids research* 1997;25:883–887. [PubMed: 9016642]
5. Lobachevsky PN, Martin RF. Iodine-125 decay in a synthetic oligodeoxynucleotide. I. Fragment size distribution and evaluation of breakage probability. *Radiation research* 2000;153:263–270. [PubMed: 10669547]
6. Lobachevsky PN, Martin RF. Iodine-125 decay in a synthetic oligodeoxynucleotide. II. The role of auger electron irradiation compared to charge neutralization in DNA breakage. *Radiation research* 2000;153:271–278. [PubMed: 10669548]
7. Linz U, Stocklin G. Chemical and biological consequences of the radioactive decay of iodine-125 in plasmid DNA. *Radiation research* 1985;101:262–278. [PubMed: 3975356]
8. Li WB, Friedland W, Jacob P, Panyutin IG, Paretzke HG. Simulation of (¹²⁵)I decay in a synthetic oligodeoxynucleotide with normal and distorted geometry and the role of radiation and non-radiation actions. *Radiation and environmental biophysics* 2004;43:23–33. [PubMed: 15042380]
9. Datta K, Jaruga P, Dizdaroglu M, Neumann RD, Winters TA. Molecular analysis of base damage clustering associated with a site-specific radiation-induced DNA double-strand break. *Radiation research* 2006;166:767–781. [PubMed: 17067210]
10. Panyutin IV, Luu AN, Panyutin IG, Neumann RD. Strand breaks in whole plasmid dna produced by the decay of (¹²⁵)I in a triplex-forming oligonucleotide. *Radiation research* 2001;156:158–166. [PubMed: 11448236]
11. Armitage B. Photocleavage of Nucleic Acids. *Chemical reviews* 1998;98:1171–1200. [PubMed: 11848929]
12. Burrows CJ, Muller JG. Oxidative Nucleobase Modifications Leading to Strand Scission. *Chemical reviews* 1998;98:1109–1152. [PubMed: 11848927]
13. Giese B. Long-distance electron transfer through DNA. *Annual review of biochemistry* 2002;71:51–70.
14. Cai, Z.; Sevilla, M., editors. *Studies of Excess Electron and Hole Transfer in DNA at Low Temperatures*. 2004.
15. Cai Z, Sevilla MD. Electron and hole transfer from DNA base radicals to oxidized products of guanine in DNA. *Radiation research* 2003;159:411–419. [PubMed: 12600244]
16. Bernhard WA, Debye MG. Electron and Hole Transfer Induced by Thermal Annealing of Crystalline DNA X-Irradiated at 4 K. *J Phys Chem B* 2000;104:7845–7851.
17. Schuster GB. Long-range charge transfer in DNA: transient structural distortions control the distance dependence. *Accounts of chemical research* 2000;33:253–260. [PubMed: 10775318]
18. Giese B. Long-distance charge transport in DNA: the hopping mechanism. *Accounts of chemical research* 2000;33:631–636. [PubMed: 10995201]
19. Boon EM, Barton JK. Charge transport in DNA. *Current opinion in structural biology* 2002;12:320–329. [PubMed: 12127450]
20. Schuster GB, Gasper SM. Intramolecular Photoinduced Electron Transfer to Anthraquinones Linked to Duplex DNA: The Effect of Gaps and Traps on Long-Range Radical Cation Migration. *Journal of the American Chemical Society* 1997;119:10.
21. Bhattacharya PK, Barton JK. Influence of intervening mismatches on long-range guanine oxidation in DNA duplexes. *Journal of the American Chemical Society* 2001;123:8649–8656. [PubMed: 11535068]
22. Kanvah S, Schuster GB. The sacrificial role of easily oxidizable sites in the protection of DNA from damage. *Nucleic acids research* 2005;33:5133–5138. [PubMed: 16157862]
23. Roll C, Ketterl C, Fazakerley GV, Boulard Y. Solution structures of a duplex containing an adenine opposite a gap (absence of one nucleotide). An NMR study and molecular dynamic simulations with explicit water molecules. *European journal of biochemistry / FEBS* 1999;264:120–131. [PubMed: 10447680]

24. Roll C, Ketterle C, Faibis V, Fazakerley GV, Boulard Y. Conformations of nicked and gapped DNA structures by NMR and molecular dynamic simulations in water. *Biochemistry* 1998;37:4059–4070. [PubMed: 9521727]
25. Giese B. Electron transfer in DNA. *Current opinion in chemical biology* 2002;6:612–618. [PubMed: 12413545]
26. Lipscomb LA, Peek ME, Morningstar ML, Verghis SM, Miller EM, Rich A, Essigmann JM, Williams LD. X-ray structure of a DNA decamer containing 7,8-dihydro-8-oxoguanine. *Proceedings of the National Academy of Sciences of the United States of America* 1995;92:719–723. [PubMed: 7846041]
27. Ito T, Rokita SE. Excess electron transfer from an internally conjugated aromatic amine to 5-bromo-2'-deoxyuridine in DNA. *Journal of the American Chemical Society* 2003;125:11480–11481. [PubMed: 13129334]
28. Ito T, Rokita SE. Criteria for efficient transport of excess electrons in DNA. *Angewandte Chemie (International ed)* 2004;43:1839–1842.
29. Schwögler A, Burgdorf LT, Carell T. Self-repairing DNA based on a reductive electron transfer through the base stack. *Angewandte Chemie - International Edition* 2000;39:3.
30. Ito T, Rokita SE. Reductive electron injection into duplex DNA by aromatic amines. *Journal of the American Chemical Society* 2004;126:15552–15559. [PubMed: 15563184]
31. Cai Z, Gu Z, Sevilla MD. Electron spin resonance study of the temperature dependence of electron transfer in DNA: Competitive processes of tunneling, protonation at carbon, and hopping. *Journal of Physical Chemistry B* 2000;104:6.
32. Messer A, Carpenter K, Forzley K, Buchanan J, Yang S, Razskazovskii Y, Cai Z, Sevilla MD. Electron Spin Resonance Study of Electron Transfer Rates in DNA: Determination of the Tunneling Constant β for Single-Step Excess Electron Transfer. *Journal of Physical Chemistry B* 2000;104:9.
33. Razskazovskii Y, Swarts SG, Falcone JM, Taylor C, Sevilla MD. Competitive Electron Scavenging by Chemically Modified Pyrimidine Bases in Bromine-Doped DNA: Relative Efficiencies and Relevance to Intrastrand Electron Migration Distances. *Journal of Physical Chemistry B* 1997;101:8.
34. Butterworth KT, Wyer JA, Brennan-Fournet M, Latimer CJ, Shah MB, Currell FJ, Hirst DG. Variation of strand break yield for plasmid DNA irradiated with high-Z metal nanoparticles. *Radiation research* 2008;170:381–387. [PubMed: 18763863]
35. Panyutin IG, Neumann RD. Sequence-specific DNA double-strand breaks induced by triplex forming 125I labeled oligonucleotides. *Nucleic acids research* 1994;22:4979–4982. [PubMed: 7800489]
36. Gaidamakova EK, Neumann RD, Panyutin IG. Site-specific strand breaks in RNA produced by (125) I radiodecay. *Nucleic acids research* 2002;30:4960–4965. [PubMed: 12434000]
37. Martin RF, Haseltine WA. Range of radiochemical damage to DNA with decay of iodine-125. *Science* 1981;213:896–898. [PubMed: 7256283]
38. Wan C, Fiebig T, Kelley SO, Treadway CR, Barton JK, Zewail AH. Femtosecond dynamics of DNA-mediated electron transfer. *Proceedings of the National Academy of Sciences of the United States of America* 1999;96:6014–6019. [PubMed: 10339533]
39. Dee D, Baur ME. Charge and excitation migration in DNA Chains. *Journal of Chemical Physics* 1974;60:20.
40. Pomplun E, Sutmann G. Is coulomb explosion a damaging mechanism for (125)IUDR? *International journal of radiation biology* 2004;80:855–860. [PubMed: 15764393]
41. Lui C-S, Hernandez R, Schuster GB. Mechanism for Radical Cation Transport in Duplex DNA Oligonucleotides. *Journal of the American Chemical Society* 2004;126:7.
42. Conwell, E., editor. *Polarons and Transport in DNA*. 2004.
43. Razskazovskii Y, Falcone JM, Taylor C, Sevilla MD. Competitive Electron Scavenging by Chemically Modified Pyrimidine Bases in Bromine-Doped DNA: Relative Efficiencies and Relevance to Intrastrand Electron Migration Distances. *Journal of Physical Chemistry B* 1997;101:8. S. S.
44. Balagurumoorthy P, Chen K, Adelstein SJ, Kassis AI. Auger electron-induced double-strand breaks depend on DNA topology. *Radiation research* 2008;170:70–82. [PubMed: 18582152]

FIG. 1. PAGE analysis of the effects of the gapped and mismatched samples

The nicked DNA control sample (*N*, Lane 1) and the gapped DNA (*G*, Lane 2) were annealed to their complementary iodine-125 containing hairpin templates prior to incubation for 2 weeks. The control samples were *MC*, without iodine-125 (Lane 3) and *MC.i*, with iodine-125 (Lane 4). The mismatched samples, were *M1*, with one CA mismatch and iodine-125 (Lane 5) and *M2*, two CA mismatches and iodine-125 (Lane 6) respectively.

FIG. 2. Analysis of the probability of breaks and frequency of breaks for gapped and mismatched DNA samples

All these samples contained ^{125}I -dC. **Fig. 2A** shows the frequency of breaks for the nicked DNA control (*N*) sample and the gapped DNA (*G*) sample. Note: The samples *N* and *G* were located 1 base and 2 bases away from the ^{125}I -dC, respectively. **Fig. 2B** shows the probability of breaks for *N* and *G*. **Fig. 2C** shows the probability of breaks for *MC.i* (control), *M1* (one CA mismatch 2 base pairs away from the initiation site - ^{125}I dCTP), and *M2* (two CA mismatches 4 base pairs away).

FIG. 3. Comparison of the effect of piperidine treatment on iodine-125 DNA damage in presence of 8-oxo-G

(-) Piperidine samples were not treated with piperidine (*8C.i* (Lane 1), *8X.i* (Lane 2), *8X* (Lane 3), *8C* (Lane 4)). Piperidine treated samples (*8C.i* (Lane 5), *8X.i* (Lane 6), *8X* (Lane 7), *8C* (Lane 8), *8xs* (Lane 9), and *8cs* (Lane 10)) were incubated at 90°C for 30min with piperidine.

FIG. 4. Probability of breaks analysis for 8-oxo-G samples

Fig. 4A shows the probability of breaks analysis for (-) piperidine samples (*8C.i* and *8X.i*) and **Fig. 4B** shows the analysis for (+) piperidine samples (*8C.i* and *8X.i*). The background had not been corrected for the 8-oxo-G in *8X.i*. **Fig. 4C** shows the analysis for (+) piperidine samples (*8C.i* and *8X.i*). The 8-oxo-G in *8X.i* is background corrected. **Fig. 4D** shows the probability of breaks for piperidine labile breaks obtained from the subtraction of *8C.i* ((-) piperidine) from *8C.i* ((+) piperidine) in control samples, and the subtraction of *8X.i* ((-) piperidine) from *8X.i* ((+) piperidine) in the experimental samples with 8-oxo-G.

FIG. 5. PAGE analysis of the effect of BrdU on ^{125}I DNA damage

All the samples (**Bc** (short oligomer, Lane 1), **Bu** (short oligomer with BrdU, Lane 2), **BC** (Lane 3), **BU** (with BrdU, Lane 4), **BU.i** (with BrdU and iodine-125, Lane 5) and **BC.i** (with iodine-125, Lane 6)) were treated with the BrdU Excision Mix for 1 hr prior to running the gel.

FIG. 6. Probability of breaks analysis for BrdU samples

The experimental sample (*BU.i*) contained a BrdU base three bases away from the site of initiation, while the control sample (*BC.i*) had a thymine base at this position. **Fig. 6A** shows the uncorrected probability of breaks analysis for *BU.i* and *BC.i*. **Fig. 6B** shows the background corrected probability of breaks analysis.

TABLE 1
DNA hairpin sequences used to assess the effect of “charge transport” barriers on ^{125}I DNA Damage

Name	Sequence ^a	Variables	
		^{125}I	Barrier
N	5' CGATGCCAGACTCCTCCTCCTCAGTTGGATGCCGTT, 3' * GCTACGGTCTGAGGAGGAGGA!GTCACCTACGGCTT	+	-
G	5' CGATGCCAGACTCCTCCTCCTCAGTTGGATGCCGTT, 3' * GCTACGGTCTGAGGAGGAGGA TCACCTACGGCTT	+	Gap
MC	5' CGATGCCAGACTCCTCCTCCTCAGTTGGATGCCGTT, 3' * GCTACGGTCTGAGGAGGAGGAGTCAACCTACGGCTT	-	-
MC.i	5' CGATGCCAGACTCCTCCTCCTCAGTTGGATGCCGTT, 3' * GCTACGGTCTGAGGAGGAGGAGTCAACCTACGGCTT	+	-
M1	5' CGATGCCAGACTCCTCCTCCTCAGTTGGATGCCGTT, 3' * GCTACGGTCTGAGGAGGAGAGTCAACCTACGGCTT	+	1 Mismatch
M2	5' CGATGCCAGACTCCTCCTCCTCAGTTGGATGCCGTT, 3' * GCTACGGTCTGAGGAGGAGAAAGTCAACCTACGGCTT	+	2 Mismatch
8C	5' CGATGCCAGACTCCTCCTCCTCAGTTGGATGCCGTT, 3' * GCTACGGTCTGAGGAGGAGGAGTCAACCTACGGCTT	-	-
8C.i	5' CGATGCCAGACTCCTCCTCCTCAGTTGGATGCCGTT, 3' * GCTACGGTCTGAGGAGGAGGAGTCAACCTACGGCTT	+	-
8X	5' CGATGCCAGACTCCTCCTCCTCAGTTGGATGCCGTT, 3' * GCTACGGTCTGAGGAGGAGXAGTCAACCTACGGCTT	-	8oxoG
8X.i	5' CGATGCCAGACTCCTCCTCCTCAGTTGGATGCCGTT, 3' * GCTACGGTCTGAGGAGGAGXAGTCAACCTACGGCTT	+	8oxoG
BC	5' CGATGCCAGACTCCTCCTCCTCAGTTGGATGCCGTT, 3' * GCTACGGTCTGAGGAGGAGGTGTCAACCTACGGCTT	-	-
BC.i	5' CGATGCCAGACTCCTCCTCCTCAGTTGGATGCCGTT, 3' * GCTACGGTCTGAGGAGGAGGTGTCAACCTACGGCTT	+	-
BU	5' CGATGCCAGACTCCTCCTCCTCAGTTGGATGCCGTT, 3' * GCTACGGTCTGAGGAGGAGBGTCAACCTACGGCTT	-	BrdU
BU.i	5' CGATGCCAGACTCCTCCTCCTCAGTTGGATGCCGTT, 3' * GCTACGGTCTGAGGAGGAGBGTCAACCTACGGCTT	+	BrdU

^a ^{32}P radiolabel; ! – nick; C – ^{125}I -dC; A – CA mismatch; AA – 2 CA mismatches; X – 8oxoG; B – BrdU