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Radiosensitization of DNA in presence of Pt(II)-based compounds*

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Abstract. X-ray irradiation of plasmid DNA in presence of platinum (II)-based compounds was carried out in order to assess the radiosensitization capabilities of these drugs. In present investigations pBR322 plasmid DNA was used to monitor the effectiveness of chosen compounds in inducing strand breaks. Samples were incubated in the presence of potential radiosensitisers: platinum (II) bromide and cis-diamminedibromoplatinum (II). The results were examined against a common cancer chemotherapy drug cis-diamminedichloroplatinum (II). It was found that platinum (II) bromide can greatly increase the levels of single- and double-strand break formation observed in the irradiated samples with respect to the samples containing platinum as a radiosensitizer only, possessing very little chemotherapeutic activity. The suggested drugs exhibit much higher level of radiosensitivity than widely used cisplatin and thus may be good candidates for cancer treatment.

1 Introduction

One of the major goals of modern radiotherapy is to develop methodologies which will increase cellular DNA damage in tumor cells using lower radiation doses such that damage to healthy cells is reduced and the side effects on patients are mitigated. The introduction of radiosensitizers into the tumour is the most successful among methods adopted nowadays. Such a treatment has been shown to be particularly beneficial in the treatment of neck, lung, pancreas or stomach cancers [1]. Accordingly, a wide range of radiosensitizing chemicals are being investigated, among which platinum-containing ones have been found to be the most successful [2,3]. It has been shown that such compounds are coordinated by the nitrogen atoms of purines in the DNA helix [2]. The mechanisms by which such radiosensitization occurs are still unclear, however recent research has highlighted that low energy electrons, which are the most abundant species created during high energy irradiations, may be as efficient in causing damage to biomolecules and DNA in particular [4–6], as the incident radiation itself. Such secondary particle damage can be initiated by different types of ionizing radiation, e.g., X-rays or γ-rays. The single and double strand breaks (SSBs and DSBs) produced by these secondary particles have been shown to increase damage levels approximately threefold, when Pt compounds are bound to DNA [6,7].

Among the most widely used cancer therapy drugs is cisplatin, which apart from being a very efficient chemotherapeutic drug, toxic to living cells [8], can be also used as a potential source of secondary particles, mainly low energy electrons and radicals, emitted by high energy radiation, mainly low energy electrons and radicals. This property of cisplatin has been investigated recently and an increase in single and double strand breaks formation was reported [9]. The enhancement of DNA damage upon resonant X-ray radiation in presence of platinum-derived complexes due to the Auger effects has also been widely investigated [10,11], showing that DNA damage increases upon Pt adducts that act as electron emitters. Apart from platinum-containing drugs [12], brominated compounds [13,14] have also been found to be efficient radio- and photosensitizers in clinical radiotherapy. Therefore, an even more efficient drug may be developed if it contains both platinum and bromine. In the present work we investigate Pt(II)-based molecules as possible candidates for cancer drugs. To our best knowledge there have been no studies on the effect of such compounds on DNA damage by X-rays. Although very little is known about biochemical interactions of such compounds with DNA,

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our present studies indicate that there is a high potential in vastly increasing the DNA damage levels upon irradiation in the presence of such compounds.

2 Methodology

2.1 Sample preparation and analysis

In order to model DNA damage, a well-known cloning vector pBR322, prepared as described previously [15], suspended in ultra-high purity (UHP) H_2O with pH adjusted to 8.0 with 1 M sodium hydroxide (NaOH) was chosen. This type of plasmid has already been widely used in irradiation studies [16,17]. NaOH was chosen to stabilize plasmid DNA under vacuum conditions as it was shown that a lack of cations in the vicinity of phosphate backbone in DNA solution prior to solvent evaporation leads to DNA strand breaks and is due to constituent water removal from DNA [18].

Two compounds containing Pt and Br were chosen to be tested as potential radiosensitisers: platinum (II) bromide ($PtBr_2$) and cis-diamminedibromoplatinum (II) ($Pt(NH_3)_2Br_2$, cis-Br-Pt). Additionally, cis-diamminedichloroplatinum (II) ($Pt(NH_3)_2Cl_2$, cis-Pt, cisplatin) was chosen to serve as a reference for $Pt(NH_3)_2Br_2$ and allows us to quantify how the addition of bromine ions affects the damage induced upon irradiation. The compounds and reagents were purchased from Sigma-Aldrich, UK. Cis-Br-Pt was synthesized according to a reaction scheme published previously [19]. All compounds were dissolved in UHP water to obtain 0.1 mM solutions. The pH of all solutions was measured and, when necessary, adjusted to 7.5–8.5 (the working range for reactions involving DNA) with 1 M NaOH. All solutions were filtrated through a 0.2 micron syringe filter. Complexing of DNA and Pt compounds of interest was performed in the following way: 1 μg (0.35 pmoles) of plasmid DNA, which contains 824 pmoles of guanine bases, was incubated with 1 nmole of each compound (binding site saturation conditions) in 20 μl of the total volume of the reaction at 37 °C for 9 h. Some pure DNA was also kept under the same conditions to serve as control samples in the irradiation experiments.

Additionally, $PtBr_2$ incubation was performed with 0.5 nmoles and 3 nmoles of the radiosensitizer and 0.35 pmoles of DNA. Samples were then subsequently subjected to X-ray radiation. In order to allow for all the sensitizing molecules present in solution to interact with DNA, the incubation times were increased for lower concentrations. For the highest concentration it was found that an incubation time of 9 h is sufficient and no changes in supercoiled DNA levels were observed at longer incubation times (data not shown). For mixtures of 0.35 pmoles of DNA with 3 nmoles of particular sensitizer, no supercoiled DNA could be detected after incubation when cis-Pt and cis-Br-Pt were used. Therefore, 1 nmole of each sensitizer was used in order to assess levels of damage upon X-ray irradiations.

Each complex was irradiated and analyzed in triplicate and the error bars shown in all figures represent the standard deviation of the population. It was observed (Fig. 1) that the long-term incubation of DNA at 37 °C introduced around 2% loss of supercoiled form of DNA. In the case of $PtBr_2$, up to 5% damage was induced in DNA samples by the presence of radiosensitizer in the incubated solutions, whereas introducing of cisplatin and bromocisplatin induced 45% and 40% of damage due to their chemical interaction with DNA, respectively, throughout the whole sample preparation procedure. For irradiation studies all samples were deposited on freshly cleaved mica, vacuum-dried at room temperature [20] with a small diaphragm pump and transferred into a vacuum chamber. Three reference samples were kept under high vacuum (HV) conditions (10^{-6} mbar) for the period of time corresponding to the largest irradiation dose during the experiment. Figure 1 shows 13% loss of the initial supercoiled DNA form: 8% due to placing samples on mica surface and additional 5% coming from the HV conditions. After irradiation all the DNA complex samples were recovered from mica with 5 μl of TE buffer and analyzed via agarose gel electrophoresis (AGE, 1.2% gels, 1 TBE, 2 V/cm, stained later with a SYBR Green I solution and de-stained with a running buffer).

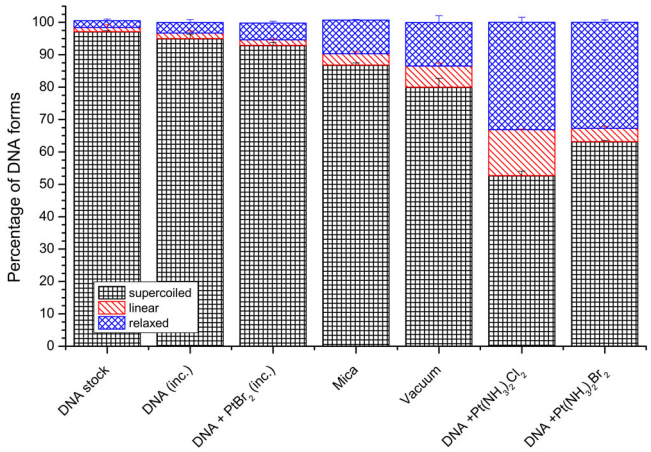

Fig. 1. Damage induced to plasmid DNA through the preparation procedure; quantities of main plasmid forms detected (from left) in: (1) stock solution, (2) stock solution incubated at 37 °C for 9 h, (3) stock solution mixed with $PtBr_2$ and incubated for 9 h at 37 °C, (4) stock solution mixed with $PtBr_2$, incubated for 9 h at 37 °C and vacuum-dried on mica, (5) stock solution mixed with $PtBr_2$, incubated for 9 h at 37 °C, vacuum-dried on mica and kept under irradiation conditions for 20 min (no irradiation), (6) stock solution mixed with $Pt(NH_3)_2Cl_2$, incubated for 9 h at 37 °C, vacuum-dried on mica and kept under irradiation conditions for 20 min (no irradiation) and (7) stock solution mixed with $Pt(NH_3)_2Br_2$, incubated for 9 h at 37 °C, vacuum-dried on mica and kept under irradiation conditions for 20 min (no irradiation).

2.2 X-ray irradiation

The DNA samples were irradiated at various doses in triplicate using X-rays. An X-ray tube with a tungsten anode was used, with a tube potential of 25 kV and a current

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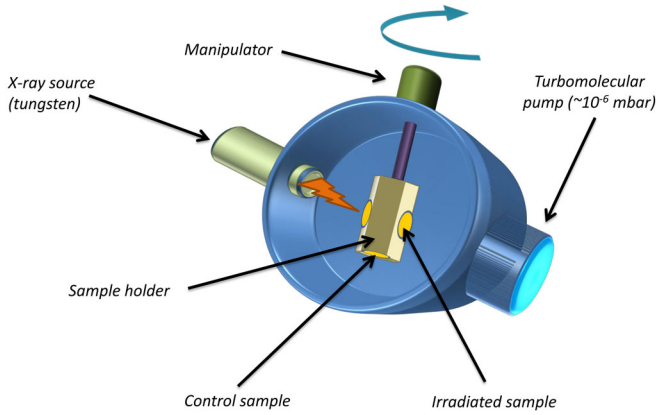

Fig. 2. Experimental setup for X-ray irradiation studies; an X-ray source used was an X-ray tube with a tungsten anode, with a tube potential of 25 kV and a current of 0.50 mA to provide a continuous X-ray spectrum up to 25 keV; delivered dose rate was approximately 11 mGy/s.

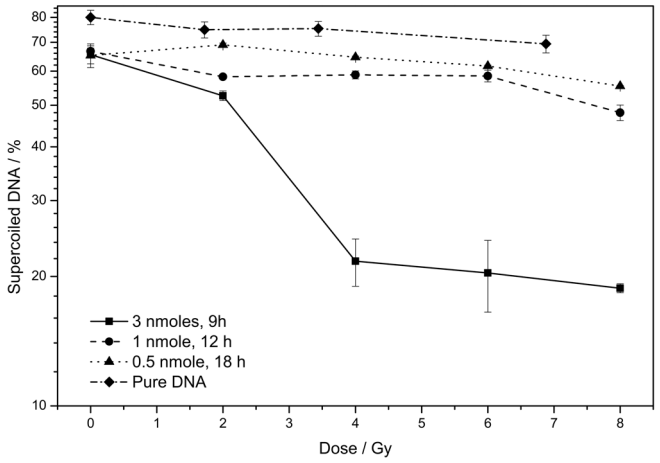

Fig. 3. Supercoiled DNA loss as a function of X-ray radiation dose in samples incubated with various concentrations of $PtBr_2$ solution for various lengths of time: 9 h, 3 nmoles (■), 12 h, 1 nmole (●) and 18 h, 0.5 nmole (▲), compared with pure DNA (♦). Curves are guides to the eye.

of 0.50 mA to provide a continuous X-ray spectrum up to 25 keV. The X-ray tube was capable of delivering a dose rate of approximately 11.5 mGy/s. Such an arrangement (depicted in Fig. 2) allowed irradiation of the samples with low energy X-rays, knowing that predominant lines in the spectrum will be L_α, L_β and M_α with energies approximately equal 8.36 keV, 9.8 keV and 1.8 keV, respectively.

3 Results and discussion

Figures 3 and 4 present the loss of supercoiled DNA form and the appearance of DSBs upon X-ray irradiation in presence of various quantities of $PtBr_2$, respectively. The level of damage obtained increases with the quantity of radiosensitizing molecules present in the sample. An increase in the levels of damage can be seen for samples incubated

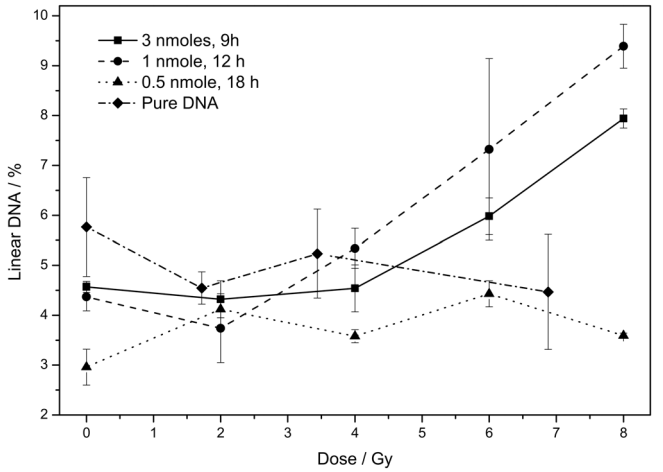

Fig. 4. Yields of DSB as a function of X-ray radiation dose in samples incubated with various concentrations of $PtBr_2$ solution for various lengths of time: 9 h, 3 nmoles (■), 12 h, 1 nmole (●) and 18 h, 0.5 nmole (▲), compared with pure DNA (♦). Curves are guides to the eye.

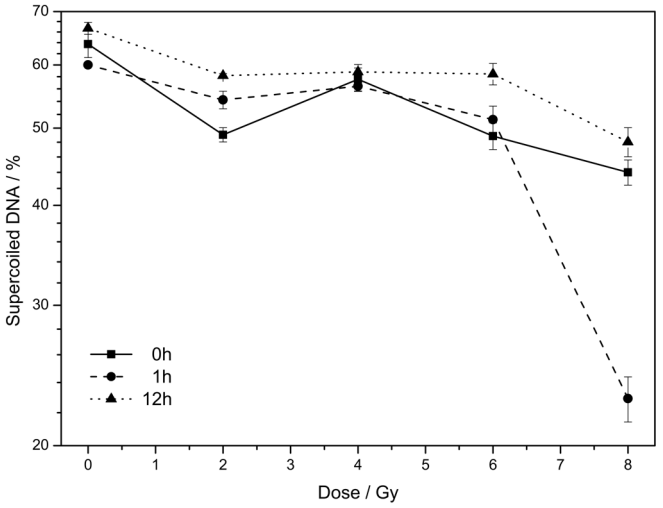

Fig. 5. Loss of supercoiled DNA form as a function of X-ray radiation dose in samples incubated with 1 nmole of $PtBr_2$ solution for 0 h (■), 1 h (●) and 12 h (▲). Curves are guides to the eye.

with $PtBr_2$ with respect to pure DNA that was kept under incubation conditions for 18 h and then irradiated.

Additionally, various incubation times for the same amount of $PtBr_2$ were tested. Samples of DNA mixed with 1 nmole of sensitizer were subjected to X-ray radiation directly after mixing, after 1 h and 12 h of incubation and subsequent vacuum-drying. The loss of supercoiled DNA form is shown in Figure 5, while the appearance of DSBs is depicted in Figure 6. It can be seen that a very small radiosensitizing effect is present after instant irradiation. The numbers of DSBs present in the sample before irradiation and formed upon photon interaction increase with incubation time at higher concentrations of $PtBr_2$, whereas when there was no incubation or the incubation time was short, the results are comparable.

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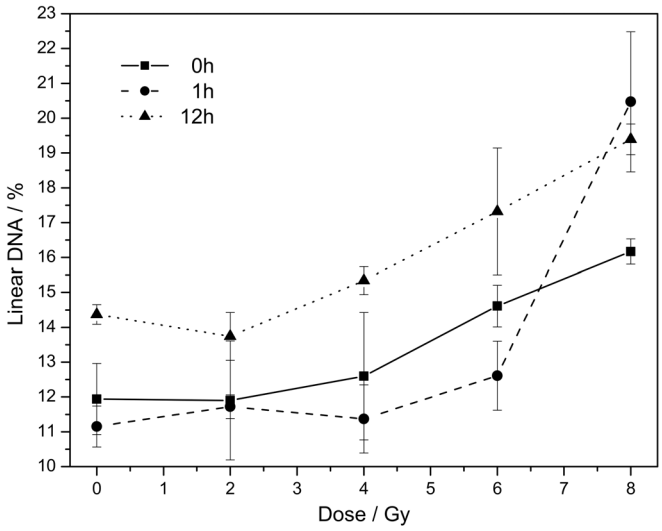

Fig. 6. Appearance of DSB as a function of X-ray radiation dose in samples incubated with 1 nmole of $PtBr_2$ solution for 0 h (■), 1 h (●) and 12 h (▲). Curves are guides to the eye.

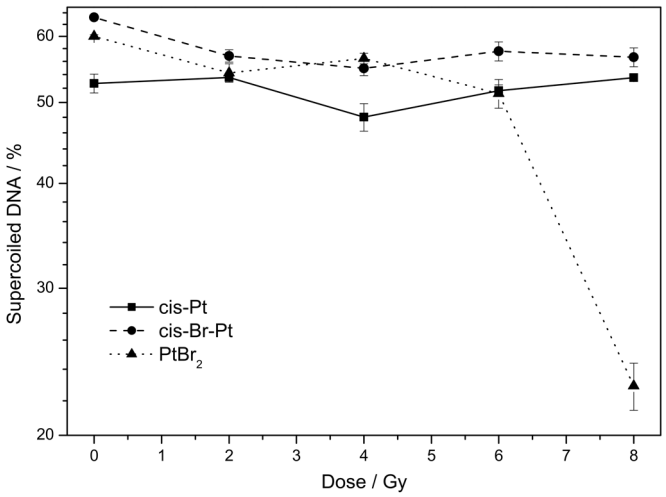

Fig. 7. Supercoiled DNA loss as a function of X-ray radiation dose for samples incubated for 12 h with 1 nmole of cis-Pt (■), cis-Br-Pt (●) and $PtBr_2$ (▲). Curves are guides to the eye.

Finally, the results obtained for DNA complexes with $PtBr_2$ were compared with widely used cisplatin and its brominated analogue. Significant levels of damage can be obtained only in case when $PtBr_2$ is used as radiosensitizer at higher doses (Fig. 7), whereas the damage levels obtained for cis-Pt and its brominated derivative result predominantly from the internal binding to DNA and possess no or a very little effect on the double-helix, when also exposed to X-rays. Moreover, an increase in the DSB formation (Fig. 8) upon irradiation was observed as a function of radiation dose only for DNA samples incubated with $PtBr_2$, whereas in two other cases DSB levels remained unchanged during irradiation.

The irradiation carried in presence of $PtBr_2$ shows significantly high levels of damage induced with respect to pure plasmid molecules (up to 60%, Fig. 3). This compound, when irradiated, also caused a considerable increase in levels of the double strand breaks (Fig. 4). Furthermore, in comparison to widely used cisplatin and its brominated analogue a higher radiosensitivity was obtained for complexes with $PtBr_2$ (Figs. 7 and 8).

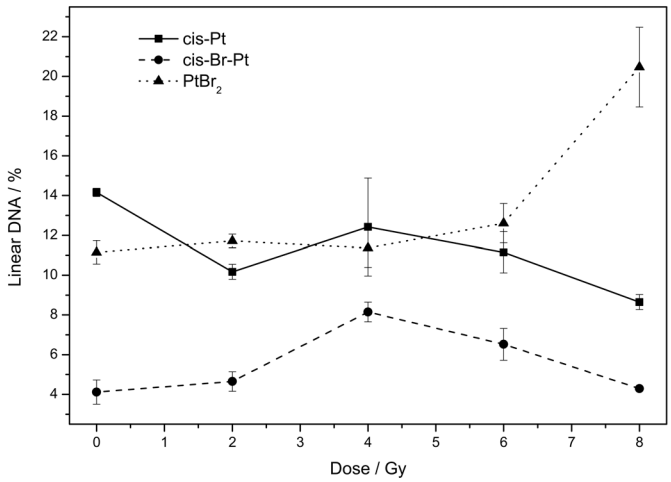

Fig. 8. Percentage amount of linear DNA as a function of X-ray radiation dose for samples incubated for 12 h with 1 nmole of of cis-Pt (■), cis-Br-Pt (●) and $PtBr_2$ (▲). Curves are guides to the eye.

Comparing our results with the ones obtained for cisplatin [11], it can be seen that for non-modified DNA the supercoiled form loss is at the level of 10% in our measurements over the whole range, whereas in case of the previously published experiments [11], the increase reaches 60%. This clearly shows that the doses of radiation used in our experiments are significantly lower, than in case of the previous experiments [11], thus no radiosensitivity was observed for DNA irradiated in presence of cis-Pt and cis-Br-Pt. Therefore, there is a huge increase of damage levels with significantly lower dose of delivered radiation for $PtBr_2$, when the loss of supercoiled DNA reaches up to 60% of the initial form for the highest concentration of the radiosensitizer (Fig. 3).

Apart from an increase of SSBs levels, a substantial increase in DSBs levels was seen for $PtBr_2$, which is an important feature for a potential radiosensitizer. For the investigated $PtBr_2$ compound the number of DSBs almost doubled after the irradiation with respect to the initial 4.5% of damage, whereas for cisplatin and its brominated analogue the damage levels remained unchanged over the irradiation period.

4 Conclusions

The mechanism by which DNA damage is induced by radiation in presence of $PtBr_2$ needs to be explored, yet it is believed that in this case the most probable pathway for DNA damage is due to the secondary particles (low energy electrons and OH radicals) that are formed upon incident high energy radiation. It has been shown

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that the 12.5 eV electrons tend to form H_2O^+ ions as a product of the ionization of the constituent gas-like water molecules [21], which may lead to further DNA strand breakage due to secondary electrons. Moreover, at higher electron energies water molecules can dissociate and produce hydroxyl radicals.

It is believed that the observed damage for $PtBr_2$ originates from bromine rather than platinum. In cases of cis-Pt and cis-Br-Pt no damage was induced upon irradiation. It is known that cis-Pt forms coordinative bonds with DNA through exchange of its chlorine atoms [22] and thus halogens are not expected to be present in close proximity of DNA. For cis-Br-Pt the mode of binding is expected to be similar. $PtBr_2$ is a hydrophobic compound, therefore hydrolysis of bromine is not expected in this case. Although the biochemical interaction of $PtBr_2$ with DNA remains unclear, it can be seen from comparison with data obtained for other compounds that it is the presence of bromine that is causing the difference. Also, the X-ray source used in the experiment does not produce emission lines that possess energy high enough to cause Auger emission from platinum. In case of non-resonant processes, the radiation doses used in the experiment are far too low for cis-Pt as seen in case of other studies [11].

Due to the positive electron affinity of bromine atom (3.363 eV), low energy electrons may easily be captured by Br and thus form negative ions. Dissociative electron attachment to $PtBr_2$ at electron energies from 0 to 10 eV has already been studied [23] and Br^- anion formation has been reported. The predominant channels for the dissociation of $PtBr_2$ molecule were found at (1) 1.2 eV and ascribed to $Br^- + PtBr$ formation and (2) 7.0 eV, where $Br^- + Pt + Br$ dissociation mechanism was assigned.

Another possible damage pathway concerns exited states of bromine atom, formed upon incident radiation [24]. These excited states may release additional electrons when colliding with low energy electrons and thus enchance the damage levels. It is also worth noticing that $M_{\alpha 1}$ emission line from the tungsten target (1.779 keV) coincides with L_I edge energy of bromine (1.782 keV) and this may cause a photoelectron emission from bromine atoms.

The way $PtBr_2$ interacts with DNA is still unknown. In order to explore the possible binding pathways we plan to investigate in the near future $PtBr_2$-DNA complexes by means of X-ray photoelectron spectroscopy (XPS).

In conclusion, although the mechanism is not yet fully understood, the present data implies that there is a potential for suggested $PtBr_2$ compound to be used as radiosensitizer in cancer therapy.

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