Nano-Sensitization under gamma rays and fast ion radiation

E PORCEL1, S LI1, N USAMI2, H REMITA3, Y FURUSAWA4, K KOBAYASHI2, C LE SECH1 and S LACOMBE1

1 Institut des Sciences Moléculaires d’Orsay (UMR 8214) Bât 351, Université Paris Sud 11, CNRS, 91405, Orsay Cedex, France.
2 Photon Factory, Institute of Materials Structure Science, High Energy Accelerator Research Organization, Oho, Tsukuba, Ibaraki 305-0801, Japan.
3 Laboratoire de Chimie Physique (UMR 8000) Bât 349, Université Paris Sud 11, CNRS, 91405, Orsay Cedex, France.
4 Research Center for Charged Particle Therapy, National Institute of Radiological Sciences, 4-9-1 Anagawa, Inage-ku, Chiba 263-8555, Japan.

E-mail: sandrine.lacombe@u-psud.fr

Abstract. The use of heavy compounds to enhance radiation induced damage is a promising approach to improve the therapeutic index of radiotherapy. In order to quantify and control the effects of these radiosensitizers, it is of fundamental interest to describe the elementary processes which take place at the molecular level. Using DNA as a probe, we present a comparison of the damage induced in the presence of platinum compounds exposed to different types of ionizing radiation. We present the results obtained with gamma rays (Linear Energy Transfer (LET) = 0.2 keV.µm⁻¹), fast helium ions He²⁺ (LET = 2.3 keV.µm⁻¹) and fast carbon ions C⁶⁺ (LET =13 keV.µm⁻¹ and LET=110 keV.µm⁻¹). The efficiency of two different sensitizers was measured: platinum based molecules (the chloroterpypyridine platinum - PtTC) and platinum nanoparticles (PtNP). These experiments show that the two sensitizers are efficiently amplifying molecular damage under photon or ion irradiation. Experiments with a radical scavenger confirmed that these damages are mediated by free radicals for more than 90%. More interestingly, the induction of complex damage, the most lethal for the cells, is amplified by a factor of 1.5 on average if platinum (PtTC and PtNP) is present. As already known, the induction of complex damages increases also with the radiation LET. So, finally, the most significant enhancement of complex damage is observed when ion radiation is combined with platinum induced sensitization.

1. Introduction

Ionizing particles deliver their energy along their tracks and induce different inelastic processes like excitations or ionizations of atoms and molecules in the irradiated material. These inelastic mechanisms result in the emission of energetic secondary electrons which can induce subsequent breaks of chemical bounds. In tissues of living organisms cell death might result as a consequence of the irradiation and this property is used for therapeutic purposes to treat tumors by radiotherapy.

In order to improve the efficiency of radiotherapy one possible way is to load the tumoral tissues with high-Z atoms. Molecules containing high-Z atoms, as for example gold salts, are used for medical purposes. Chemotherapy protocols frequently use platinum salts such as cis-platinum. Our group has
previously shown that platinum salts enhance DNA damage and cell death induced by radiation (X-rays or fast ions) [1]. An inner shell ionization of the heavy atom by the ionizing particles or by the secondary electrons emitted along the track will mainly generate an Auger cascade and result in the amplification of electron emission. Subsequently, it will induce an enhancement of the water radiolysis in the vicinity of the heavy atom [2]. The local release of a dense cluster of water radicals is very efficient to cause complex damage in the surrounding molecules. This promising strategy is however limited by the localization of the sensizers in the tumor. Recently, some developments in nanoscience opened new perspectives by using nanoparticles, whose surfaces can be functionalized by tumor specific molecules [3, 4]. Nanoparticles made of heavy atoms are also of interest to achieve the radiosensitization of biological tissues. They are presently the subject of an increasing number of studies [5-8]. Most of these studies are focused on the effects of X-rays. However, such highly penetrating photons induce severe damage before (in the entrance channel) and behind the tumors. An alternative and superior approach consists of using fast ions as ionizing radiation [9].

In this work, we compare the efficiency of sensitization induced by platinum salts and platinum nanoparticles when gamma rays (LET = 0.2 keV.µm\(^{-1}\)) or fast helium and carbon ions (LET = 2.3, 13 and 110 keV.µm\(^{-1}\)) are used as ionizing radiation sources. Studies at the molecular level shall help to understand the elementary mechanisms. Recent microscopy experiments have clearly shown that the radiosensitization is not limited to the compartment of nuclear DNA [10, 11]. We use, however, DNA as a good probe to quantify simultaneously the induction of simple and complex damage. In particular, the induction of complex (nanometric) damage is of high interest because they induce cell death. The goal of this work is thus to quantify the molecular damage induced by combining radiation of different LET and platinum induced radiosensitization.

2. Experimental section

2.1. DNA samples
Plasmid DNA pBR322 (supplied by Toyobo or Promega) is extracted from E. Coli. They are supplied in Tris EDTA (TE) buffer (10 mmol.L\(^{-1}\) Tris-HCl –pH 7.6– and 1 mmol.L\(^{-1}\) ethylenediaminetetraacetic acid –EDTA–). This buffer is chosen because of the presence of chlorine, which mimics the medium in living cells. The molecule is a circular double-stranded DNA of 4361 base pairs (2.8 \(\times\) 10\(^6\) Dalton per molecule). Three forms of plasmid can be found depending on the degradation level. In the intact form of the supercoiled plasmid one strand has more turns as compared to the other. This results in a topological constraint that can be removed only by strand breaks. A single strand break (SSB) relaxes the constraint and the plasmid becomes “circular”. A double strand break DNA (DSB) consists of two breaks occurring face to face - within about ten base pairs - in the two opposite strands separated by 2 nm and the plasmid becomes open in a form called “linear”. The three forms can be easily separated by electrophoresis performing a migration in agarose gel. Prior to irradiation, DNA samples contain more than 95% supercoiled, 5% circular, and no linear forms.

2.2. Chloroterpiridine platinum
This molecule is provided by FLUKA –Sigma Aldrich Chemie Gmbh Schnelldorf Germany– in powder form and used without any further purification. A solution of PtTC was prepared by dilution in ultra pure water. The concentration was determined by spectrophotometry at the wave length \(\lambda = 278\) nm with an absorbance coefficient \(\varepsilon = 25100\) mol\(^{-1}\).L.cm\(^{-1}\) [12]. Dilutions were made to obtain a final solution of a concentration of 4.23 \(\times\) 10\(^{-5}\) mol.L\(^{-1}\).

2.3. Platinum nanoparticles
Platinum nanoparticles (PtNP) were synthesized by radiolysis [13]. The synthesis is described elsewhere [14]. Briefly, the PtNP were made from platinum salts [Pt(NH\(_3\))\(_4\)]Cl\(_2\), H\(_2\)O –Sigma Aldrich– diluted in ultra pure water. A polymer, the poly acrylic acid (PAA) C\(_3\)H\(_4\)O\(_2\)C\(_3\)H\(_4\)O\(_2\)Na –Sigma Aldrich–, was added to stop aggregation and stabilize the aggregate. The solution prepared at a
platinum concentration of $10^{-4}$ mol.L$^{-1}$ was irradiated by gamma rays to induce the production of reducing species (e$^-$, H$^+$). The dose needed for complete reduction of platinum (+II) in platinum (0) was 1000 Gy. After aggregation of platinum (0), homogeneous nanoparticles of 3 nm in diameter and containing 1000 platinum atoms on average were obtained [14].

2.4. Preparation of the samples

Solutions of DNA molecules containing platinum were prepared with a ratio of one platinum atom per 15 DNA phosphate groups (one platinum atom for 7 or 8 base pairs on average). The experiments with PtTC and PtNP were performed with the same atomic concentration of platinum in DNA.

An electrostatic binding is established between DNA (negative) and PtTC (positive) [12]. At this concentration, a negligible fraction of PtTC remains free in solution and a complete saturation of DNA by PtTC is observed [15]. A similar type of electrostatic binding is expected for PtNP.

In order to investigate the role of the indirect effects (mediated by the solvent radicals), some experiments were performed in the presence of the free radical scavenger, Dimethylsulfoxide (DMSO), at a concentration of 1 mol.L$^{-1}$.

Finally, the samples containing 500 ng of DNA (1 µL) were diluted in 12 µL of TE buffer at room temperature. The PtTC and PtNP solutions were added to DNA at a same final concentration of $5.6 \times 10^{-6}$ mol.L$^{-1}$ (1 platinum for 15 DNA phosphate groups). In order to keep the concentration in salts (TE buffer) similar in all the experiments, the total volume of the samples was adjusted to 18 µL with pure water (used as the solvent of the sensitizers and DMSO).

2.5. Irradiation

The DNA solutions were placed in Eppendorf vessels of 0.5 mL. Irradiations of these samples were respectively performed with gamma rays, and fast ions (He$^{2+}$ and C$^{6+}$). The experiments were performed with pure DNA, DNA loaded with PtTC and DNA loaded with PtNP. The irradiations were conducted under atmospheric conditions at room temperature. Samples were irradiated with increasing doses ranging from 0 to 400 Gy.

The gamma irradiation of DNA was performed in a panoramic $^{60}$Co source located at Paris Sud University (Orsay - France). The average energy of the photon beam is 1.25 MeV and the LET is 0.2 keV.µm$^{-1}$. Samples were irradiated at the maximum dose rate (33 Gy.min$^{-1}$).

The irradiation of DNA by fast ions were performed at the Heavy Ion Medical Accelerator (HIMAC) located in Chiba, Japan. DNA was irradiated by ions of different Linear Energy Transfer (LET): He$^{2+}$ at the entrance channel of the beam (Energy = 150 MeV/uma, LET = 2.3 keV.µm$^{-1}$), C$^{6+}$ at the entrance of the beam (Energy = 290 MeV/uma, LET = 13 keV.µm$^{-1}$) and C$^{6+}$ at the maximum of the Spread Out Bragg Peak – SOBP – (Initial energy = 290 MeV/uma, average LET = 110 keV.µm$^{-1}$). The dose rates were equal to 4 Gy.min$^{-1}$ for helium and 15 Gy.min$^{-1}$ for carbon.

2.6. Analysis

After irradiation, the samples were analyzed by agarose gel electrophoresis. The gel consisted of 1% agarose in a buffer: Tris acetate EDTA (40 mmol.L$^{-1}$ Tris, 20 mmol.L$^{-1}$ acetate and 1 mmol.L$^{-1}$ EDTA). After 2h30 of migration at room temperature (10 V.cm$^{-1}$), the gel is stained with Ethidium Bromide at 0.3 µg.mL$^{-1}$ and placed under Ultra Violet light (312 nm). The DNA fluorescence was recorded with a Charged Coupled Device (CCD) camera. An image analysis software (Image Quant) was used to determine the integrated fluorescence of the three bands corresponding to the three plasmid conformations. Considering Ethidium bromide binds 1.47 times less on supercoiled plasmids $–S–$ than in relaxed $–R–$ and linear $–L–$ forms, the normalized fractions of supercoiled plasmids $–S–$, relaxed $–R–$ and linear $–L–$ forms were obtained and the normalized fractions of single ($SSBpl$) and double strand breaks ($DSBpl$) per plasmid were finally calculated according to [16]:

\[ \text{Norme : } N = S \times 1.47 + R + L \]
The final outcome is the number of SSB and DSB per plasmid versus the dose. No significant artefacts due to the binding of PtTC or PtNP to DNA were found in electrophoresis. Furthermore, the different radiosensitizers did not cleave the plasmids without irradiation.

3. Results and discussion

3.1. Amplification of radiation effects

The radiosensitizing properties of platinum compounds were characterized by measuring the induction of the DNA breaks: single strand breaks (SSB) and double strand breaks (DSB). DSB is the signature of nanometer scale damage, difficult to repair by the cells, while SSB corresponds to a simple bond breaking. For this purpose, plasmid DNA is used as a sensitive probe to quantify and to characterize simultaneously this damage induced by radiation, with and without sensitizers.

The number of SSB and DSB in DNA plasmids loaded with or without platinum nanoparticles or platinum atoms induced by gamma rays (LET = 0.2 keV.µm⁻¹), by helium ions He²⁺ (LET = 2.3 keV.µm⁻¹), by carbon ions C⁶⁺ (LET = 13 keV.µm⁻¹), and by carbon ions C⁶⁺ (LET = 110 keV.µm⁻¹) have been measured as a function of the dose. Some results, obtained with gamma rays and helium ions are presented in figure 1. The calculations related to these measurements are summarized for all types of irradiation in table 1.

For every radiation condition, the number of breaks (SSB, DSB) increases with the dose and follows a linear law. This finding suggests that a single event is able to induce a double strand break. In the presence of platinum (salts and nanoparticles), an enhancement of DNA damage is observed. The measurement is in agreement with previous work [14, 17].
Figure 1. SSB (1a and 1b) and DSB (2a and 2b) per plasmid induced by gamma rays (energy = 1.25 MeV, LET = 0.2 keV,\(\mu\)m\(^{-1}\)) irradiation (a: left column) and He\(^{2+}\) (energy = 150 MeV/\(\mu\)ma, LET = 2.3 keV,\(\mu\)m\(^{-1}\)) (b: right column) in the pure DNA (open triangle), in the presence of PtNP (open diamond) or PtNP + DMSO (open circles) and in the presence of PtTC (open square).

The yields of SSB and DSB induced to DNA are defined as the number of breaks induced per plasmid and per gray. They correspond to the slopes of the dose–response curves as presented in figure 1 for gamma rays and helium ions. The yields are reported in table 1 for all types of irradiation (gamma rays, helium ions and carbon ions) in the case of pure DNA, DNA+PtTC and DNA+PtNP samples.

In order to make a quantitative analysis of the effects of the radiosensitizers, the sensitizing factors – SF – are defined as the ratio of the number of SSB (respectively DSB) per plasmid and per Gray induced in DNA loaded with platinum divided by the number of SSB (respectively DSB) per plasmid and per Gray obtained in pure DNA (see table 1 - column 4):

\[
SF_{(SSB, DSB)} = \frac{m_{SSB, DSB,(Pr)}}{m_{SSB, DSB,(Pr=0)}}
\]
Table 1. Yields of SSB and DSB (slopes $m$) induced by gamma rays (LET = 0.2 keV,µm$^{-1}$), He$^{2+}$ ions (LET = 2.3 keV,µm$^{-1}$), C$^{6+}$ ions from entrance (LET = 13 keV,µm$^{-1}$) and C$^{6+}$ ions from SOBP (LET = 110 keV,µm$^{-1}$) in pure DNA, DNA + PtTC and DNA+ PtNP (with or without DMSO).

| Irradiation source (LET) | Samples          | Induction of breaks (per plasmid per gray) | Sensitizing Factor | $m_{SSB}$/$m_{DSB}$ |
|-------------------------|------------------|--------------------------------------------|-------------------|--------------------|
|                         |                  | $m_{SSB}$ $\times 10^{-4}$ | $m_{DSB}$ $\times 10^{-5}$ | SF$_{SSB}$ | SF$_{DSB}$ |                  |
| **Gamma $^{60}$Co** (LET = 0.2 keV/µm) | Pure DNA | 86 ($\pm$1.9) | 5.2 ($\pm$0.2) | 165 |
|                         | DNA+PtTC | 139.5 ($\pm$2.4) | 7.9 ($\pm$0.3) | 1.6 ($\pm$0.1) | 1.5 ($\pm$0.2) | 177 |
|                         | DNA+PtNP | 120.1 ($\pm$1.7) | 7.8 ($\pm$0.3) | 1.4 ($\pm$0.1) | 1.5 ($\pm$0.1) | 154 |
|                         | DNA+PtNP+DMSO | 8.1 ($\pm$0.2) | 0.7 ($\pm$0.1) | | |
| **Ions He$^{2+}$** (LET = 2.3 keV/µm) | Pure DNA | 57.4 ($\pm$0.5) | 8.6 ($\pm$0.4) | 67 |
|                         | DNA+PtTC | 97.8 ($\pm$0.8) | 13.2 ($\pm$0.5) | 1.7 ($\pm$0.1) | 1.5 ($\pm$0.2) | 74 |
|                         | DNA+PtNP | 69.3 ($\pm$1.2) | 13.3 ($\pm$0.7) | 1.2 ($\pm$0.1) | 1.5 ($\pm$0.2) | 52 |
| **Ions C$^{6+}$** (LET = 13 keV/µm) | Pure DNA | 53.1 ($\pm$0.6) | 8.6 ($\pm$0.5) | 62 |
|                         | DNA+PtTC | 88 ($\pm$1) | 13.8 ($\pm$0.6) | 1.7 ($\pm$0.1) | 1.6 ($\pm$0.2) | 64 |
|                         | DNA+PtNP | 73 ($\pm$3) | 18.3 ($\pm$0.8) | 1.4 ($\pm$0.1) | 2.1 ($\pm$0.3) | 40 |
|                         | DNA+PtNP+DMSO | 7.2 ($\pm$1.9) | 1.7 ($\pm$0.1) | | |
| **Ions C$^{6+}$** (LET = 110 keV/µm) | Pure DNA | 34.5 ($\pm$0.2) | 9.5 ($\pm$0.1) | 36 |
|                         | DNA+PtTC | 50.9 ($\pm$2.6) | 14.9 ($\pm$0.9) | 1.5 ($\pm$0.1) | 1.6 ($\pm$0.1) | 29 |
|                         | DNA+PtNP | 43.2 ($\pm$2.3) | 12.3 ($\pm$0.4) | 1.3 ($\pm$0.1) | 1.3 ($\pm$0.1) | 35 |
|                         | DNA+PtNP+DMSO | 4.4 ($\pm$0.3) | 1.6 ($\pm$0.1) | | |

3.2. Role of direct and indirect effects

Some experiments were performed with DNA containing PtNP in the presence of a radical scavenger (DMSO) (see figure 1 for gamma rays radiation). DMSO is an efficient scavenger, which, at this concentration (1 mol.L$^{-1}$), totally inhibits the activity of water radicals. It is used to quantify the role of HO$\cdot$ radicals. It should be noted that the chlorine contained in the TE buffer is also a radical scavenger but, at this concentration, it only mimics the scavenging conditions of living cells [18]. The calculated yields of DNA breaks (in the presence of PtNP) due to direct and indirect effects with gamma rays and C$^{6+}$ are reported in table 2. A large decrease in the strand breaks yields is observed. It confirms previous results of the group obtained with carbon ions [14, 17]. These measurements show that the enhancement of SSB and DSB is mostly mediated by HO$\cdot$ radicals for more than 90%. The effect of direct processes, including the direct ionization of the biomolecule by electrons or ions produced in the medium, and the effect of low energy electrons attachment, is very low. It indicates that the early stage processes initiated by the radiation and amplified by sensitizers ends in the major part with the
production of water radicals prior to the induction of biological damage. These early stage processes have been described elsewhere [14].

Table 2. Yields of SSB and DSB per plasmid and per gray due to direct and indirect effects induced by gamma irradiation (LET = 0.2 keV.µm⁻¹) and ions C⁶⁺ irradiation (LET = 13 keV.µm⁻¹) in presence of PtNP.

|                  | Gamma rays | C⁶⁺ ions |
|------------------|------------|----------|
| **SSB**          |            |          |
| direct effect    | 7 %        | 10 %     |
| indirect effect  | 93 %       | 90 %     |
| **DSB**          |            |          |
| direct effect    | 9 %        | 9 %      |
| indirect effect  | 91 %       | 91 %     |

3.3. Influence of LET on the sensitizing effect

From table 1 we see that the induction of SSB decreases when LET increases. On the contrary, DSB increase with LET. As a result, the ratio \( m_{SSB} / m_{DSB} \) (table 1, last column) decreases when LET increases. This result confirms that the complexity of the damage increases with the ionizing density (LET) of the radiation [19]. The gamma rays have high energy and low LET resulting in dispersed ionizations events. Hence it is likely then that it will cause only single strand breaks in the DNA helix. In contrast, high LET charged particles such as carbon ions cause highly localized ionizations, which favor the induction of double-strand breaks.

This finding is also observed in the presence of sensitizers (PtTC and PtNP). In order to better compare the efficiencies of PtTC and PtNP, we report in figure 2 the sensitizing factors obtained with different types of radiation. For all types, the sensitizing factor of PtTC is close to 1.5 (± 0.1) for SSB and DSB (SF\(_{SSB}\) and SF\(_{DSB}\)). For PtNP, the sensitizing factors vary from 1.3 up to 2.1.

The goal of radiotherapy and radiosensitization is to eradicate cancer cells. We thus focus the discussion on the induction of complex molecular damage (DSB) which are the most lethal for the cells. As shown in figure 2, the efficiency of platinum molecules and nanoparticles (PtTC and PtNP) to induce complex damage –DSB– is found to be close to 1.5 on average for incident photons and ions. With the number of DSB increasing with LET, it is clearly the combination of ion radiation and radiosensitizers wich causes the highest rate of complex damage.
Figure 2. Sensitizing factors for SSB (a) and DSB (b) induced in DNA loaded with PtTC or PtNP for different LET (see values in table 1).

We notice that the best sensitizing effect is reached ($\text{SF}_{\text{DSB}} = 2.1$) when PtNP are combined with $\text{C}^{6+}$ –LET $= 13$ keV.$\mu$m$^{-1}$ – radiation. This implies that the nanoparticles are better electron emitters than isolated metal atoms (PtTC), because of auto-amplified electron cascades specific to nanoparticles [14].

In the case of incident helium ions (and gamma rays), there is no difference between PtNP and PtTC effects. This indicates that the nanoparticles are better activated by electrons emitted along the high LET particle track.
Interestingly, the efficiency of PtNP is getting lower (SF<sub>DSB</sub> = 1.3) when the LET of carbon ions increases to 110 keV.µm<sup>-1</sup>. This decrease of sensitization for high LET carbon ions compared to medium LET carbon ions can be attributed to the well known mechanisms of HO recombination. Indeed, the production of hydroxyl radicals in the nanometric volume around the nanoparticles is expected to increase because of the ionizing density of the incident particles and because of the amplification induced by PtNP. The proximity of HO species favors the recombination into less reactive H<sub>2</sub>O<sub>2</sub>. As a result, the induction of molecular damage decreases due to the decreasing HO concentration. This phenomenon has been observed for cells irradiated by high LET radiation such as Argon ions [20-22]. In the presence of PtNP, this saturation occurs for lower LET [17].

4. Conclusion

The present experiments show that the presence of heavy atoms, in the form of salts or nanoparticles, induces a strong amplification of molecular damages (SSB, DSB) when gamma rays or fast ions (at different LET values) are used as ionizing radiation. The induction of complex damage being higher for high LET radiations, the most advantageous solution to enhance cell death is certainly the combination of sensitizers with fast ions. Finally, even though, the amplification at the Bragg peak was found lower than at the entrance, this factor is close to 1.3 which corresponds to an amplification of 30% of the molecular damage.

The use of a radical scavenger in some experiments confirms that the induction and the amplification of the molecular damage induced by incident ions and photons are mostly related to the production of free radicals in the medium (indirect processes). The direct processes (including direct breaks induced by electrons or photons) contribute with less than 10%.

The sensitization induced by nanoparticles is close to the effects of the salts (except for specific conditions of irradiation with carbon ions of medium LET). But, the main advantage of the NP stems from the possibility to functionalize their surface with tumor targeting molecules [3, 4]. The use of nanoparticles can thus improve the selective enrichment of cancer cells in sensitizing agents.

Finally, the combination of radiosensitizers and atomic ions could significantly improve the therapeutic index of radiotherapy. Regarding the results presented here, there is a strong need to develop and test new nanoparticles, different in composition, size, and shape. There is also a strong need for molecular simulations to interpret and predict their effects with a view to better controlling the association of nanoparticles with fast ion radiation. In the end, the choice from a large number of radiosensitizers would open up prospects for personalized cancer treatment.

References

[1] Kobayashi K, Usami N, Porcel E, Lacombe S and Le Sech C 2010 Enhancement of radiation effect by heavy elements Mutation Research-Reviews in Mutation Research 704 123-31
[2] Laster B H, Thomlinson W C and Fairchild R G 1993 Photon-Activation of Iododeoxyuridine - Biological Efficacy of Auger Electrons Radiat. Res. 133 219-24
[3] Kong T, Zeng J, Wang X P, Yang X Y, Yang J, McQuarrie S, McEwan A, Roa W, Chen J and Xing J Z 2008 Enhancement of radiation cytotoxicity in breast-cancer cells by localized attachment of gold nanoparticles Small 4 1537-43
[4] Li X, Zhou H, Yang L, Du G, Pai-Panandiker A S, Huang X and Yan B 2011 Enhancement of cell recognition in vitro by dual-ligand cancer targeting gold nanoparticles Biomaterials 32 2540-5
[5] Hainfeld J F, Slatkin D N and Smilowitz H M 2004 The use of gold nanoparticles to enhance radiotherapy in mice Physics in Medicine and Biology 49 N309-N15
[6] Mesbahi A 2010 A review on gold nanoparticles radiosensitization effect in radiation therapy of cancer Reports of Practical Oncology & Radiotherapy 15 176-80
[7] Mowat P., Mignot A., Rima W., Lux F., Tillement O., Roulin C., Dutreix M., Bechet D., Huger S., Humbert S., Barberi-Heyob M., Aloy M.T., Armandy E., Rodriguez-Lafrasse C., Le Duc G., Roux S. and Perriat P. 2011 In vitro radiosensitizing effects of ultrasmall gadolinium based particles on tumour cells Journal of Nanoscience and Nanotechnology 11 7833

[8] Butterworth K T, Wyer J A, Brennan-Fournet M, Latimer C J, Shah M B, Currell F J and Hirst D G 2008 Variation of strand break yield for plasmid DNA irradiated with high-Z metal nanoparticles Radiat. Res. 170 381-7

[9] Kraft G 2003 ed. F Currell: Kluwer Academic Publishers Chapter 5: Radiobiological effects of highly charged ions

[10] Bridot J-L, Dayde D, Riviere C, Mandon C, Billotey C, Lerondel S, Sabattier R, Cartron G, Le Pape A, Blondiaux G, Janier M, Perriat P, Roux S and Tillement O 2009 Hybrid gadolinium oxide nanoparticles combining imaging and therapy Journal of Materials Chemistry 19 2328-35

[11] Usami N, Furusawa Y, Kobayashi K, Lacombe S, Reynaud-Angelin A, Sage E, Wu T D, Croisy A, Guerquin-Kern J L and Le Sech C 2008 Mammalian cells loaded with platinum-containing molecules are sensitized to fast atomic ions International Journal of Radiation Biology 84 603-11

[12] Jennette K W, Lippard S J, Vassiliades G A and Bauer W R 1974 Metallointercalation reagents. 2-hydroxyethanethiolato(2,2',2'-terpyridine)-platinum(II) monocation binds strongly to DNA by intercalation Proc Natl Acad Sci U S A 71 3839-43

[13] Belloni J, Mostafavi M, Remita H, Marignier J L and Delcourt M O 1998 Radiation-induced synthesis of mono- and multi-metallic clusters and nanocolloids New Journal of Chemistry 22 1239-55

[14] Porcel E, Liehn S, Remita H, Usami N, Kobayashi K, Furusawa Y, Le Sech C and Lacombe S 2010 Platinum nanoparticles: a promising material for future cancer therapy? Nanotechnology 21 7

[15] Le Sech C, Takakura K, Saint-Marc C, Frohlich H, Charlier M, Usami N and Kobayashi K 2001 Enhanced strand break induction of DNA by resonant metal-innershell photoabsorption Canadian Journal of Physiology and Pharmacology 79 196-200

[16] Spotheim-Maurizot M, Charlier M and Sabattier R 1990 DNA Radiolysis by Fast-Neutrons International Journal of Radiation Biology 57 301-13

[17] Usami N, Kobayashi K, Furusawa Y, Frohlich H, Lacombe S and Le Sech C 2007 Irradiation of DNA loaded with platinum containing molecules by fast atomic ions C60 and Fe26+ International Journal of Radiation Biology 83 569-76

[18] Jones G D D, Milligan J R, Ward J F, Calabrojones P M and Aguilera J A 1993 Yield of Strand Breaks as a Function of Scavenger Concentration and Let for Sv40 Irradiated with He-4 Ions Radiat. Res. 136 190-6

[19] Newman H C, Prise K M, Folkard M and Michael B D 1997 DNA double-strand break distributions in X-ray and alpha-particle irradiated V79 cells: Evidence for non-random breakage International Journal of Radiation Biology 71 347-63

[20] Scholz M and Kraft G 1996 Track structure and the calculation of biological effects of heavy charged particles Advances in space research : the official journal of the Committee on Space Research (COSPAR) 18 5-14

[21] Taucher-Scholz G, Heilmann J and Kraft G 1996 Induction and rejoining of DNA double-strand breaks in CHO cells after heavy ion irradiation Advances in space research : the official journal of the Committee on Space Research (COSPAR) 18 83-92

[22] Gervais B, Beuve M, Olivera G H and Galassi M E 2006 Numerical simulation of multiple ionization and high LET effects in liquid water radiolysis Radiation Physics and Chemistry 75 493-513