ESRF	**Experiment title:** *Radiosensitization of human cells by platinum for hadrontherapy purpose: impact of metal speciation*	**Experiment number**: EC 405
Beamline: BM 30B	**Date of experiment**: from: 10/12/2008 to: 15/12/2008	**Date of report**: 10/02/2009
Shifts: 15	**Local contact(s)**: Isabelle Alliot	*Received at ESRF:*

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Introduction

A growing number of scientific reports demonstrate that the presence of a metal in human cells, and particularly platinum, efficiently enhances radiation-induced cell damage and death. This effect is particularly significant when using charged particle beams. Such studies have potential application in **anti-cancer hadrontherapy**, particularly for **radioresistant tumours**. However the physical, chemical and biological mechanisms governing this enhanced radiosensitivity remain to be characterized. First information on the mechanisms of platinum radiosensitizing have been obtained using carboplatin (1-3), cisplatin (4) and terpyridine-platinum (PtTC) (5-6). In the latter experiments, the sensitizing effect was mainly attributed to an increase of the $OH^{\cdot}$ free radical production following the relaxation of the ionized platinum atoms. Recently, Pt nanoparticles showed the same radiosensitizing properties on in vitro assays on plasmid DNA (S. Lacombe, unpublished results). **Platinum speciation** has not been addressed clearly during these experiments. However it may have a major influence since oxidative processes are implicated in the efficiency of the treatments.
The aim of the present study was to understand the influence of platinum speciation in radiosensitization mechanisms. For this purpose cell were loaded with Pt(0), Pt(II) or Pt(IV), and Pt speciation was followed after alpha or gamma irradiation, at doses ranging from 0.2 to 8 Gy.

Experimental methods

A549 cells (ATCC, CCL-185, human alveolar carcinoma) were exposed during 24h to sublethal concentrations of Pt(0) (Pt nanoparticles, "nanoPt"), Pt(II) (cisplatin *i.e.* *cis*-$PtCl_2(NH_3)_2$ or "cisPt"; terpyridine-platinum or "PtTC") or Pt(IV) ($PtCl_4(NH_3)_2$ or "Pt3.2.4"). Alpha irradiations were performed directly on the cell monolayers, using the single ion irradiation tool of Laboratoire Pierre Süe (7), modified in order to permit the irradiation of large cell culture surfaces in a few minutes. The total deposited dose was estimated to be 0.1-0.2 Gy. For gamma irradiations, cells were collected, centrifuged and the cell pellet was exposed to 2, 4 or 8 Gy. Directly after irradiation (t0), or after 2h of recovery in complete cell culture medium at 37°C / 5% CO2 (t+2h), cells frozen in liquid nitrogen and freeze-dried. Lyophilisates were pressed into 5 mm pellets (8). X-ray absorption spectra (XANES and EXAFS) of these pellets were collected at Pt L_{III}-edge in fluorescence mode, in a helium cryostat in order to avoid sample speciation evolution which may be caused by the beam.

Results

References

XANES spectra of reference compounds are given in figure 1. As expected, the main peak (white line) of CisPt and PtTC (Pt(II)) are only shifted by 0.2 and 0.8 eV, respectively, as compared to the white line of NanoPt. The white line of Pt(IV) is shifted by 1.5 eV as compared to the white line of NanoPt. Just after the white line, the first EXAFS oscillations are quite different, which suggests that information may be available from this part of spectra.

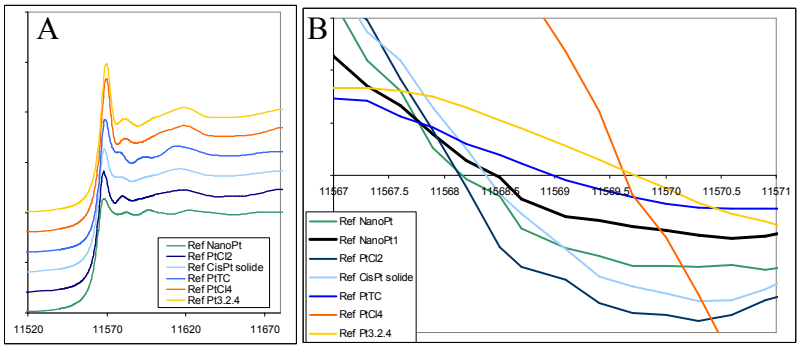

Figure 1. XANES spectra (A) and their first derivative (B) related to reference compounds

NanoPt

Cell exposure to NanoPt did not induce any modification of Pt speciation, neither in non irradiated cells nor in irradiated cells. This means that NanoPt are inert in cells or that their diameter is too important for surface modifications to be observed without being hidden by XAS signal of the bulk particle. It may be interesting to analyse the speciation of smaller Pt nanoparticles, which may be more efficiently accumulated in cells, more efficient as radiosensitizers and for which surface modification may be observable by XAS.

Pt(II) and Pt(IV)

6 to 24 h after cell exposure to Pt, without irradiation, Pt speciation inside the cell is different from the initial Pt speciation. Pt(IV) is reduced inside cells: the position of the white line is shifted from -0.4 eV in cell samples as compared to Pt3.2.4 reference. Both Pt(II) compounds are oxidized: the positions of the white line are shifted from 0.5 eV for PtTC and from 0.8 eV for CisPt (Figure 2 and table 1). In all these cell samples, the overall shape of spectra are modified as compared to the corresponding reference spectrum: oscillations are attenuated, and the first oscillation after XANES main peak almost totally disappears in CisPt and Pt3.2.4-exposed cells.

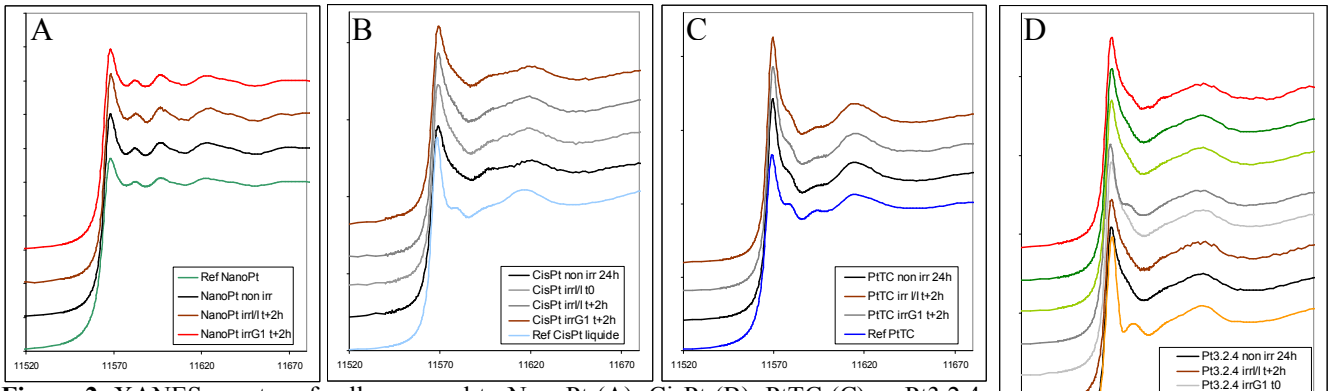

Figure 2. XANES spectra of cells exposed to NanoPt (A), CisPt (B), PtTC (C) or Pt3.2.4 (D). Cells were exposed to Pt compounds for 24h (non irr 24h), then irradiated with alpha particles (irr I/I), irradiated with gamma rays at the dose of 2Gy (irrG1), 4Gy (irrG2) or 8 Gy (irrG3) and directly frozen (t0) or incubated for 2h in culture medium (irr I/I t+2h).

A	NanoPt	CisPt	PtTC	Pt3.2.4
Ref	11568.2	11568.4	11569	11569.7
non irr 24h	11568.2	11569.2	11569.3	11569.3
irr I/I t0		11569.1		
irr I/I t+2h	11568.4	11569.25	11569.4	11569.5
irr G1 t0				11569.4
irr G1 t+2h	11568.4	11569.25	11569.5	11568.7
irr G2 t0				11569.4
irr G2 t+2h				11569.5
irr G3 t+2h				11569.3

B	NanoPt	CisPt	PtTC	Pt3.2.4
Ref	11568.2	11568.4	11569	11569.7
non irr 24h	0	0.8	0.3	-0.4
irr I/I t0		0.7		
irr I/I t+2h	0.2	0.85	0.4	-0.2
irr G1 t0				-0.3
irr G1 t+2h	0.2	0.85	0.5	**-1**
irr G2 t0				-0.3
irr G2 t+2h				-0.2
irr G3 t+2h				-0.4

Table 1. Energy corresponding to the maximum of the white line (A), and shift between this energy in sample spectra as compared to reference spectra (B). Energies are expressed in eV.

We did not detect any speciation modification after Pt exposure and alpha irradiation of cells (Figure 2, table 1). However, after irradiation with 2 Gy of gamma-rays, a stricking modification of Pt3.2.4 speciation was detected when cells were irradiated and then allowed to recover for 2h in cell culture medium (Pt3.2.4 irrG1 t+2h, Figure 2D). The shape of the spectrum corresponding to this sample is

different from the other spectra of the same series: a post-peak is observable juste after the white line, the maximum of absorption of the white line is reduced as compared to the other samples. The position of the white line is shifted from -1 eV as compared to the reference spectrum. In this sample, Pt is thus reduced. This modification is not observed when cells are directly frozen after irradiation or when cells are allowed to recover after irradiation with 4 Gy or 8 Gy. Our hypothesis is that after irradiation with 4 Gy or 8 Gy, damage is too important and cells stop their metabolism and concommitantly their ability to modify Pt speciation. Irradiation with 2 Gy allows cells to maintain their metabolic activities or even amplifies these activities, and during the 2 h of recovery after irradiation Pt(IV) is partly reduced to Pt(II).

Conclusions and perspectives

As demonstrated by Hall *et al.* (9), cell metabolism induces a change of speciation of Pt(II) and Pt(VI) through a reduction process. Here, we report a preliminary observation showing that ionising irradiation may enhance this effect when a low dose of spartly ionising radiation (gamma rays) is applied. Futher investigations are required, particulary to correlate speciation change with irradiation dose and Linear Energy Transfert.

References

1. Yang L, Douple EB, O'Hara JA, Crabtree RA, Eastman A (1995) Radiat Res. 144(2): 230-6
2. Yang LX, Douple EB, Wang HJ (1995) Int J Radiat Oncol Biol Phys. 33(3): 641-6
3. Yang LX, Douple E, Wang HJ (1995) Int J Radiat Biol. 68(6): 609-14
4. Corde S, Biston MC, Elleaume H, Estève F, Charvet AM, Joubert A, Ducros V, Bohic S, Simionovici A, Brochart T, Nemoz C, Renier M, Troprès I, Fiedler S, Bravin A, Thomlinson W, Le Bas JF, Balosso J (2002) Radiat Res. 158(6): 763-70.
5. Usami N, Furusawa Y, Kobayashi K, Frohlich H, Lacombre S and Le Sech C (2006) Int J Radiat Biol. 81(7): 515-22.
6. Usami N, Kobayashi K, Furusawa Y, Frohlich H, Lacombre S and Le Sech C (2007) Int J Radiat Biol. 83(9): 569-76.
7. Khodja H, Daudin L, Hanot M, Hoarau J, Carrière M, Gouget B (2006) Radiat Res 166(4): 670-1.
8. Carrière M, Proux O, Milgram S, Thiebault C, Avoscan L, Barré N, Den Auwer C, Gouget B (2008) JBIC 13(5): 655-62.
9. Hall MD, Foran GJ, Zhang M, Beale PJ, Hambley TW (2003) JACS Communications 125: 7524-25.