Cellular Distribution and Oxidation State of Platinum (II) and Platinum (IV) Antitumour Complexes in Cancer Cells

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Introduction

The anticancer activity of platinum (IV) complexes has been recognised since the discovery of the activity of cisplatin by Rosenberg and colleagues [1, 2]. While many platinum (IV) compounds have been investigated for activity, only three have entered clinical trials, and none have been registered thus far. It is generally accepted that reduction to platinum (II) is essential for the anticancer activity of platinum (IV) complexes to be affected (Fig. 1). The kinetic inertness of platinum (IV) complexes means that there is increased opportunity for the complex to arrive at the cellular target intact. Modifying the axial ligands of a platinum (IV) complex alters its lipophilicity and reduction potential and thus its ability to enter tumour cells before being reduced to yield the active platinum (II) drug.

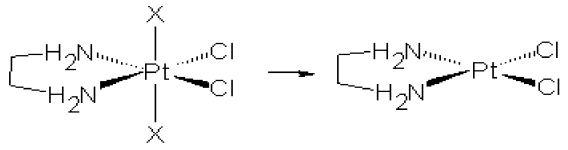


FIG. 1. The reduction of Pt(IV) to Pt(II).

The mechanism and rates of reduction of platinum (IV) complexes by endogenous biomolecules *in vitro* have been extensively investigated [3], yet the distribution of platinum drugs in tumour cells is not known. The platinum oxidation states (II) and (IV) (d_8 and d_6, respectively) do not have "electronic handles," which makes direct monitoring of the oxidation state *in vivo* difficult. X-ray absorption near-edge spectroscopy (XANES) has been used to provide information about the average oxidation state of metal ions in biological systems [4], but few x-ray absorption fine structure (XAFS) or XANES investigations of Pt drugs have been reported, and none in biological systems have been reported [5-7].

In approaching the problem of cellular uptake and distribution, a measurement technique was selected that was sensitive enough to detect low concentrations of Pt present in the cells at submicrometer resolution. In addition to this, we aimed to avoid using platinum drug conjugates in order to observe the localisation of the actual Pt drugs. Synchrotron radiation induced x-ray emission (SRIXE) allows for the accurate detection of elements down to parts-per-million (ppm) levels and allows for 2-D mapping of elemental distribution within the cell to be imaged. Since SRIXE is an elemental detection technique, the Pt drug uptake can be observed without modification of the amine ligands.

Methods and Materials

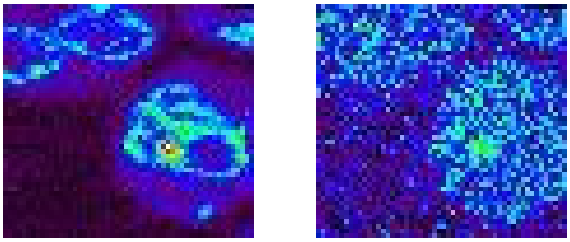
The platinum complexes *cis*-$[PtCl_2(NH_3)_2]$, *cis*-$[PtCl_4(NH_3)_2]$, *cis,trans,cis*-$[PtCl_2(OAc)_2(NH_3)_2]$, and *cis,trans,cis*-$[PtCl_2(OH)_2(NH_3)_2]$ were prepared as previously described [8-11]. Parental human ovarian carcinoma A2780 cells were treated at an effective concentration of 20 μM with each of the Pt complexes for 24 h, then washed, harvested, mounted on Formvar® film supported by a Teflon® frame, frozen in liquid N_2-cooled isopentane, and freeze-dried. Thin-sectioned samples were prepared by fixing a cell pellet in Spurr's resin and microtoming the pellet to obtain 1-μM sections [12].

Hard x-ray zone-plate-based microprobe SRIXE experiments were performed on beamline 2-ID-D at the APS. Elements analyzed were Ca, Cl, Cu, Fe, K, Ni, P, Pt, S, and Zn. Whole cells were analysed by using a 13.4-keV monochromatic x-ray beam focused to a 1×0.3-μm spot. Suitable single cells were scanned within a scan area of 20×20 μm. The SRIXE maps were collected by using 0.5-μm steps and scanning for a period of 3 s per point. Analysis of the thin-sectioned cell was performed with the beam reduced to a 0.3×0.3-μm spot, and an area of 16×16 μm was analysed. Emission was at each point for 10 s.

Micro-XANES spectra were collected from 11.45 to 11.65 keV by initially locating a single cell as described above for SRIXE analysis. The region of highest Pt concentration was identified, and XANES spectra were collected at three points in this region.

Results

The SRIXE map of the elemental distribution of a cisplatin-treated sectioned cell is shown in Fig. 2. After 24 h, the Pt is localised in the region that is coincident with the maximum zinc concentration — the region that is assumed to be the cell nucleus [13]. There is a lower concentration of Pt in the surrounding cytoplasm, and no other regions of Pt localisation are observed, including the cell membrane. While Pt is localised only in the nucleus, Ca and Cu can be observed localised in small cellular compartments.



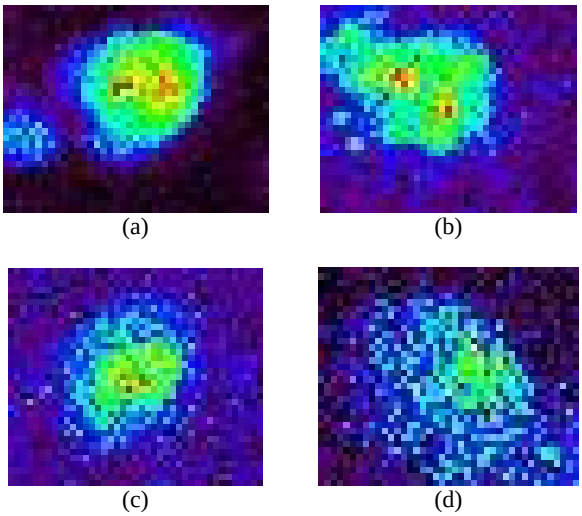
(a) (b)

FIG. 2. Elemental maps of (a) Zn and (b) Pt collected from a sectioned A2780 cell treated with cis-*[$PtCl_2(NH_3)_2$] (cisplatin).*

The SRIXE maps of the whole cells treated with the platinum complexes *cis*-[$PtCl_4(NH_3)_2$], *cis,trans,cis*-[$PtCl_2(OAc)_2(NH_3)_2$], *cis,trans,cis*-[$PtCl_2(OH)_2(NH_3)_2$], and cisplatin are shown in Fig. 3. After 24 h, the Pt is localised in the nuclear region of the cells, with a lower concentration in the surrounding cytoplasm.

The only reported investigation employing SRIXE was undertaken by Talarico [16] at 11.6 keV, to compare the cellular uptake of cisplatin and an organoamido Pt(II) analogue in human ovarian 2008 cells. No subcellular localisation was detected for either compound by Talarico.

The observed localisation of Pt in the nucleus after 24 h is in accordance with the previous observation of Khan et al. [14], but in contrast to Molenaar et al. [15], who detected no irreversible accumulation in the nucleus, and Talarico [16], who observed no subcellular localisation. Pt(IV) complexes are more inert, and a greater portion of the drug may arrive in the nucleus intact before reduction and binding to DNA. It is also possible that differences between cell lines employed in other studies are partly responsible for this disparity.



(a) (b) (c) (d)

FIG. 3. Elemental maps of Pt obtained from A2780 cells treated with (a) cis-[$PtCl_2(NH_3)_2$], (b) cis-[$PtCl_4(NH_3)_2$], (c) cis,trans,cis-[$PtCl_2(OAc)_2(NH_3)_2$], and (d) cis,trans,cis-[$PtCl_2(OH)_2(NH_3)_2$].

The micro-XANES spectra of all treated cells display a Pt L3 edge because of the Pt present in the cells. The maxima in the first derivative of the XANES spectra reveal the edge energy (Fig. 4). The Pt signal for cells treated with *cis*-[$PtCl_4(NH_3)_2$] and *cis,trans,cis*-[$PtCl_2(OAc)_2(NH_3)_2$] display a single peak in the first derivative in a manner similar to cisplatin, suggesting that the majority of the drug is reduced after 24 h. Although the spectrum for *cis,trans,cis*-[$PtCl_2(OH)_2(NH_3)_2$] is noisier because of the lower Pt signal, it appears to display two peaks in the first derivative, separated by approximately 2 eV.

Experiments in our research group have shown that the Pt edges for Pt(II) and Pt(IV) complexes lie 2 eV apart and that the Pt(II) and Pt(IV) oxidation states can be distinguished in a solution containing a mixture of Pt(II) and Pt(IV) complexes in the first derivative of the XANES spectrum because of the peak separation of 2 eV [17]. The first derivative of the XANES spectra collected from a *cis,trans,cis*-[$PtCl_2(OH)_2(NH_3)_2$]-treated cell displays a similar spectrum, supporting the conclusion that Pt(IV) is present along with Pt(II) in the cell after 24 h of exposure. The ratio of the peak height to the XAFS region differs for Pt(II) and Pt(IV) [17], and in Fig. 4(d), this too is consistent with the presence of Pt(IV).

This observation is significant since one of our goals is to design Pt(IV) complexes that remain intact until they are in the cell. The results reported here are the first detection of platinum (IV) drugs within cells and confirm the suggestion that Pt(IV) does survive until it enters the cell [18].

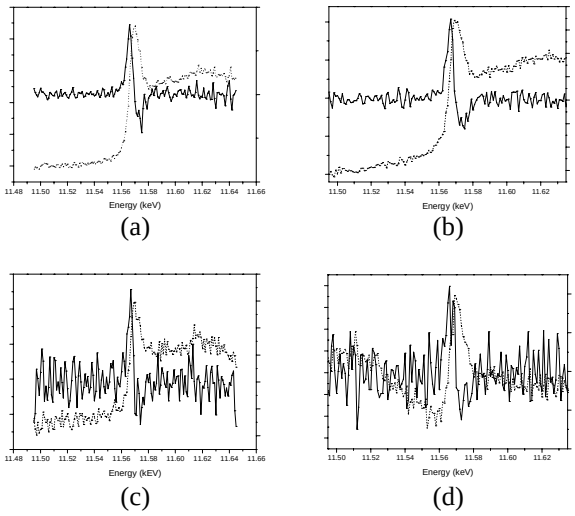


FIG. 4. The micro-XANES spectra (solid line) and first derivative (dotted line) of A2780 cells treated with (a) cis-$[PtCl_2(NH_3)_2]$, (b) cis-$[PtCl_4(NH_3)_2]$, (c) cis,trans,cis-$[PtCl_2(OAc)_2(NH_3)_2]$, and (d) cis,trans,cis-$[PtCl_2(OH)_2(NH_3)_2]$.

Discussion

In conclusion, microprobe SRIXE is an excellent method for detecting Pt drugs in whole and sectioned cells, and it is a valuable tool for obtaining insight into the cellular distribution of Pt drugs. Pt(IV) complexes appear to be distributed in a similar fashion to Pt(II) drugs, consistent with the way the majority of administered Pt(IV) drugs are reduced. This is confirmed by microprobe XANES spectra that display Pt(II) spectra, but Pt(IV) can also be detected in cells treated with the more inert *trans*-dihydroxo complex.

Acknowledgments

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