Communication

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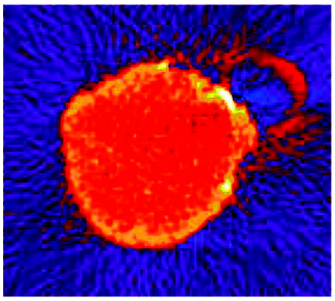
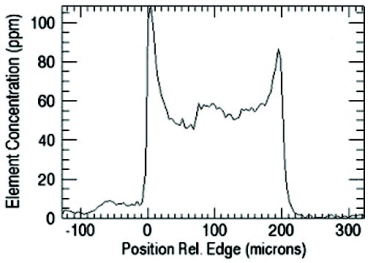
*J. Am. Chem. Soc.*, **2007**, 129 (44), 13400-13401• DOI: 10.1021/ja076281t • Publication Date (Web): 12 October 2007








Pt distribution

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Published on Web 10/12/2007

# Elemental Tomography of Cancer-Cell Spheroids Reveals Incomplete Uptake of Both Platinum(II) and Platinum(IV) Complexes

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The limited penetration of cytotoxic drugs into tumors, a major component of multicellular resistance, is a significant contributing factor to the limited effectiveness of cancer chemotherapy.[1−3] Clinically important drugs such as doxorubicin diffuse only 40−100 $\mu$m from blood vessels and reach a fraction of the viable cells that make up a solid tumor.[4,5] The multicellular tumor spheroid model is an *in vitro* system that has been extensively used to study the extent of anticancer drug penetration.[6,7] However, the techniques used to study penetration of platinum compounds are usually indirect and semiquantitative at best, mainly due to the inability to directly visualize these compounds in tissue. For example, the extent of cell killing in spheroids has been used to ascertain the degree of penetration of cisplatin through the spheroid volume;[8,9] cell sorting techniques utilizing radiolabeled complexes have been used to reconstruct the concentration gradients of cisplatin[10] and tetraplatin[11] throughout spheroid volumes, and a fluorescent Pt porphyrin complex has been used to monitor time-dependent penetration into spheroids.[12] However, these approaches sacrifice either tissue morphology or drug structure. X-ray fluorescence microtomography provides a means by which elemental distribution in virtual slices through a sample can be imaged and has been used to study the internal elemental distributions of a variety of samples,[13−15] including a brief study of spheroid samples.[16]

Highly reactive anticancer agents, including cisplatin, will suffer from rapid reaction with cells close to the surface of a tumor spheroid, and this is likely to limit the extent of their diffusion through the spheroid. Pt(IV) complexes are substantially more inert and therefore have the potential to penetrate further into the tumors while remaining largely intact. A number are in varying stages of clinical development including satraplatin which is being considered for FDA approval for use in hormone-refractory prostate cancer.[17−19] Herein we present the results of the first microtomographic study of the distribution of cisplatin and three Pt(IV) complexes in spheroids to determine the ability of this method to reveal the variation of Pt distribution for Pt(II) and Pt(IV) complexes.

Following treatment with Pt complexes for 24 h, individual whole DLD-1 human colon carcinoma spheroids were fixed and stored in neutral buffered formalin fixative. Individual spheroids were removed from formalin immediately prior to analysis and mounted onto a goniometer head. Spheroids were analyzed using a 13.45 keV monochromatic X-ray beam focused to a 4−5 $\mu$m spot (GeoSoilEnviroCARS sector at the Advanced Photon Source, Argonne, IL) to give elemental images in $\mu$g/cm$^2$. Images in element weight fraction (e.g., ppm) were computed by dividing the $\mu$g/cm$^2$ value in each pixel by the beam height and assuming the density of each pixel was that of water (i.e., 1 g/cm$^3$). The detection limit for Pt was determined to be near 10 ppm. Radial distribution profiles for each tomogram were produced by determining the concentration profile through the center of the spheroid at 1° intervals. The central portion of each profile was expanded or contracted so that each ray had the same total length. These were averaged to produce a single profile of Pt concentration through the spheroid. More complete experimental details are provided in the Supporting Information.

Each of the Pt complexes studied produced similar distribution patterns in spheroids following a 24 h incubation, with enrichment of Pt in the outer region, corresponding to the outer proliferative leaflet,[20] and uniform distribution deeper within the spheroid. The exterior Pt content was approximately double that of the interior Pt content for each of the samples. Figure 1 shows representative examples of the Pt distribution in spheroids treated with each of the Pt complexes for 24 h. Cu and Zn profiles collected simultaneously showed no surface concentration (e.g., Supporting Information, Figure S1). Studies using $^{14}$C[ethane-1,2-diamine] complexes suggest that the majority of the Pt observed in the microtomography experiments is that bound intracellularly to cell macromolecules such as DNA and proteins.[21] Unbound Pt is expected to have been washed out during preparation of the spheroid samples (fixation and brief storage in formalin). As such, information regarding the total penetration of Pt complexes into the spheroids can only be inferred, since the concentration of Pt that reached the inner regions of the spheroids but remained unbound could not be quantified. However, since the spheroids were treated for 24 h, the bound Pt will represent most accurately the fraction with cytotoxic potential.

These results indicate that cisplatin and the Pt(IV) complexes are able to penetrate and bind to the central regions of the spheroid. However the peripheral, actively dividing cells accumulate a larger portion of the drugs, leaving a smaller amount to penetrate more deeply. The spheroid periphery is analogous to tumor tissue closest to the vessel and the central region is analogous to avascular, hypoxic zones. Thus one may infer that the decreased intracellular drug concentration in avascular tumor regions may contribute to a decrease in drug efficacy and clinical resistance. Higher doses of the Pt complexes may be required to enhance penetration into central, quiescent regions of the spheroid. Previous studies with tetraplatin[11] have shown that with higher drug doses, retention of tetraplatin becomes more uniform throughout the spheroid.

The findings presented herein are consistent with previous studies using other methods, which found that cisplatin,[8,9] carboplatin,[9] and tetraplatin[11] penetrate efficiently into spheroids following incubation times of up to 2 h. A porphyrin−Pt complex was found to penetrate

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10.1021/ja076281t CCC: $37.00 

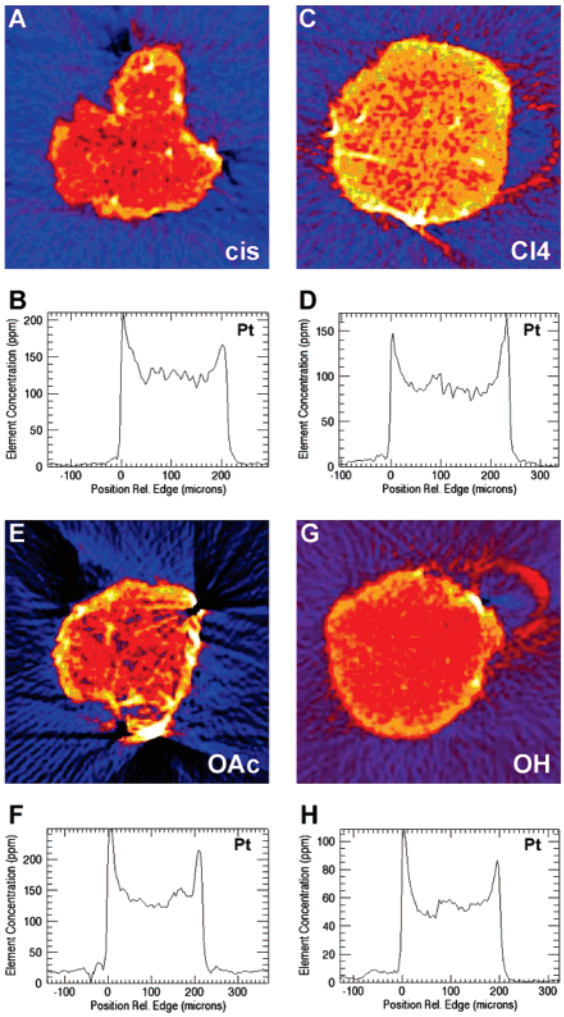


***Figure 1.*** Pt distribution and corresponding average concentration profiles through representative spheroids treated with cisplatin (cis) (*cis*-[$PtCl_2(NH_3)_2$]), 50 $\mu$M, 24 h (A, B); *cis*-[$PtCl_4(NH_3)_2$] (Cl4), 50 $\mu$M, 24 h (C, D); *cis*,*trans*,*cis*-[$PtCl_2(OAc)_2(NH_3)_2$] (OAc, OAc = $CH_3COO^-$), 200 $\mu$M, 24 h (E, F); *cis*,*trans*,*cis*-[$PtCl_2(OH)_2(NH_3)_2$] (OH), 200 $\mu$M, 24 h (G, H).

throughout spheroids within 24 h.[12] Studies of peritoneal rat tumors found that the periphery of the tumors had higher Pt concentrations, with a concentration gradient toward the center of the tumor following intraperitoneal administration of cisplatin. In contrast, following equimolar treatment with carboplatin, only low Pt concentrations were observed on the periphery of the tumors, and Pt was undetectable in the center of the tumor. When dosed at significantly higher concentrations of carboplatin, Pt distribution throughout the tumor was fairly homogeneous, though still at low concentrations.[22,23]

The profiles for spheroids treated with the Pt(IV) complexes reveal somewhat lower levels of Pt accumulation than do cisplatin treated spheroids, consistent with our observations of cellular uptake of Pt(II) and Pt(IV) complexes.[24] In all cases, a higher accumulation in the cells closer to the surface is observed. While these Pt(IV) complexes do not offer improved penetration of spheroids, we believe this is due to greater accumulation of the complexes tested by cells closer to the surface, either because of consequent depletion of the complex or because these cells are actively cycling.

Here we have shown for the first time that X-ray fluorescence microtomography is a suitable technique for imaging the distribution of Pt drugs within multicellular tumor spheroids. In the present study this has revealed that there is no significant difference between the distributions of the range of Pt(II) and Pt(IV) complexes investigated, suggesting that complexes that are more resistant to cellular uptake may be needed to ensure more complete distribution throughout a tumor.

**Acknowledgment.** T.W.H. thanks the Australian Research Council and R.C. thanks Cancer Research U.K. for funding. This work was also supported by the ASRP, which is funded by the Commonwealth of Australia under the MNRF Programme. GeoSoilEnviroCARS is supported by the National Science Foundation and Department of Energy. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences.

**Supporting Information Available:** Experimental details, Cu and Zn profiles, and a Pt profile for a control spheroid. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA076281T