

ICP - Mass Spectrometry

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New Research Evaluating Cisplatin Uptake in Ovarian Cancer Cells by Single Cell ICP-MS

Introduction

Cisplatin, carboplatin, and oxaliplatin are the most widely used of platinum-

based cancer chemotherapy drugs in the Western world. Cisplatin's effectiveness is due to its ability to bind to the DNA, resulting in DNA-platinum (Pt) adducts, which bend the DNA. The cells must then repair the DNA damage, otherwise DNA replication is blocked resulting in cell death¹.

Many cancers are initially sensitive to platinum-based treatment, but patients frequently relapse with tumors displaying resistance to further cisplatin therapy¹. Cisplatin drug resistance is due to three major molecular mechanisms: increased DNA repair, increased cytosolic inactivation, and altered cellular accumulation. Decreased cellular uptake or increased cellular export of cisplatin constitutes the mechanisms involved in altered cellular accumulation¹.

Cellular uptake of cisplatin is related to tumor burden, meaning low intracellular cisplatin levels are associated with a decreased tumor response to cisplatin. There is a need to analyze the uptake and distribution of cisplatin at the level of a single cell to determine whether treatment is effective. More cisplatin imported into cells results in increased DNA damage and more frequent cell death. Understanding the cellular uptake of cisplatin will allow for the development of new strategies and therapies to increase tumor sensitivity to cisplatin.

The problem with studying cisplatin uptake is the methodology, as represented in Figure 1. Traditionally, a population of cells has been treated with cisplatin, and the total amount of platinum in the cell population is measured. The cells are digested, and a Pt concentration is obtained by techniques such as atomic absorption spectroscopy (AAS) or inductively coupled plasma-mass spectrometry (ICP-MS). However, the significance of the concentration is unclear, leading to several questions:

- What does the concentration mean in terms of cells?
- Do all cells have the same intracellular cisplatin concentration?
- Or do some cells have high concentrations while others have little or no cisplatin?

The downside of this traditional approach is that the Pt concentration represents the total population of cells rather than individual cells. Therefore, the distribution and individual cellular variation of cisplatin uptake is not represented by traditional methods. In reality, cellular uptake of cisplatin most likely varies in response, but until now, there have not been effective methods to evaluate its uptake.

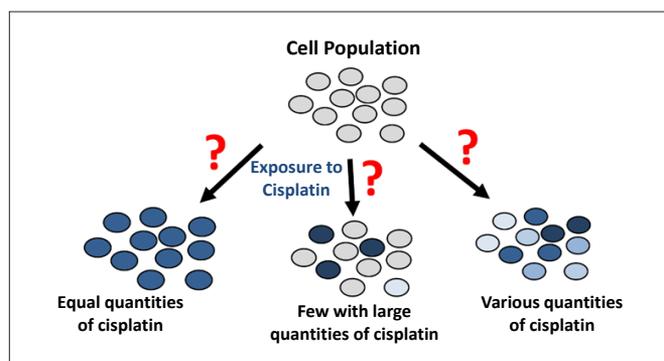


Figure 1. Possible results of cisplatin uptake by a population of cells.

A new, emerging technique has been developed that allows for the quantitation of metal at the level of a single cell: Single Cell ICP-MS (SC-ICP-MS). SC-ICP-MS is based on the Single Particle ICP-MS (SP-ICP-MS) technique which allows for the evaluation and quantitation of nanoparticles in solution. Both techniques are based on the ability to measure discrete signals generated from a single cell (or a nanoparticle) when they enter the plasma. The metal content in each cell is ionized, producing an ion plume that is measured with the enabled fast data acquisition of 100,000 data points per second, allowing the metal content within individual cells to be quantified down to attogram (ag) per cell levels. The resulting information yields increased information in comparison to traditional methods to measure cisplatin uptake in cells.

This work describes how SC-ICP-MS is used to explore cellular uptake of cisplatin at the single cell level.

Experimental

Cell Culture and Cisplatin Treatment

The ovarian cancer cell lines A2780 and A2780/CP70 were used in all experiments. Cells were grown in RPMI 1641 media (Gibco™) supplemented with 10% fetal bovine serum (FBS, Gibco™), insulin (Sigma-Aldrich™), l-glutamine (Gibco™), and pen/strep (Gibco™) under 5% CO₂ at 37 °C. For serum starvation experiments, cells were plated and allowed to attach to the dishes. The media was removed and replaced with serum-free media for approximately 18 hours after which the media was replaced with regular media to begin cisplatin treatment. Cisplatin was re-suspended at 1 mg/mL in sterile saline and vigorously shaken for 30 minutes prior to treatment. Figure 2 shows the schematic of the sample preparation. Cells were treated with 30 μM cisplatin for a time course where samples were collected at 1, 2, 4, and 8 hours post-treatment. For analysis, cells were washed twice with phosphate-buffered saline (PBS) and collected using the nonenzymatic cell dissociation solution Cellstripper (Corning™). Cells were centrifuged at 500 g-force for 10 minutes. The supernatant was discarded, and the cells were resuspended in 1 mL PBS, filtered through a 70 μm nylon mesh, and quantitated by hemocytometer counting. Cells were diluted in PBS to a final concentration of 100,000 cells/mL and kept on ice until injection.

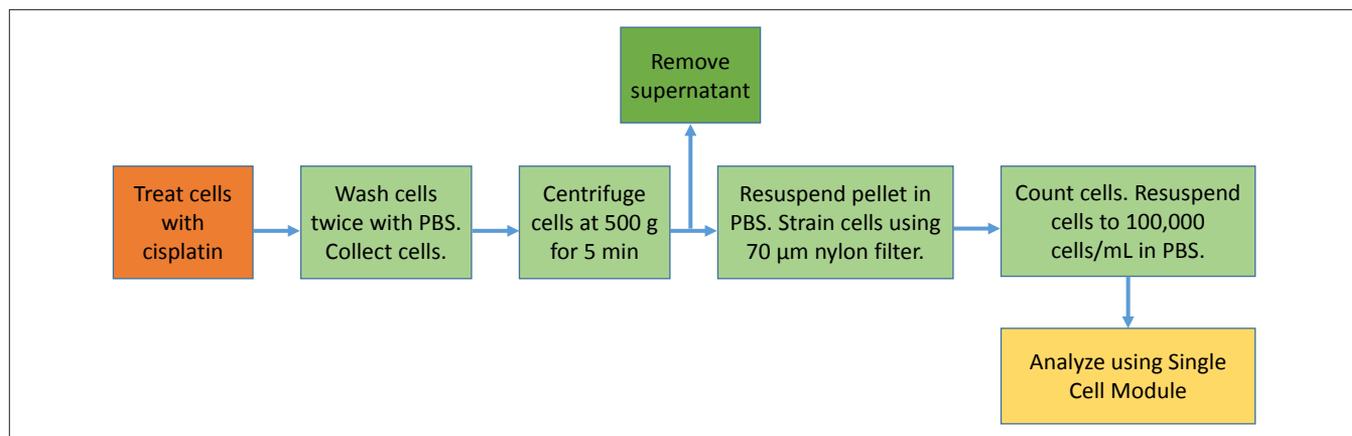


Figure 2. Schematic showing sample preparation steps.

Instrumentation

Analyses were performed with PerkinElmer's NexION® ICP-MS using the Syngistix™ Single Cell Application Software Module for data collection and processing, as shown in Figure 3. Instrumental conditions are shown in Table 1, with the NexION being tuned daily for optimum performance and sensitivity. The Single Cell Sample Introduction Kit (Part No. N8150031) was used for sample introduction to the NexION. The kit consists of a high-efficiency nebulizer (Part No. N8142046) and the proprietary, new Asperon™ spray chamber designed specifically for optimal transport of cells to the plasma (Part No. N8152493). Since cells (1-100 μm) are larger than droplets which typically pass through conventional spray chambers (< 2 μm), a new spray chamber was designed for optimal performance.

Ionic platinum standards were prepared in PBS to matrix match the cell sample. A standard curve was generated using 1, 2, and 3 ppb platinum standard. Transport efficiency was determined using 60 μm gold nanoparticles in PBS.

Results and Discussion

The cells chosen for this study are the ovarian cancer cell lines A2780 and A2780/CP70, which are good models for developing the single cell method since the molecular mechanism for resistance is altered by cisplatin uptake. A2780 is a cisplatin-sensitive cell line, while the A2780/CP70 line is cisplatin resistant. This resistance was developed by exposing the A2780 parental cell line to increasing doses of cisplatin. The

Table 1. ICP-MS Conditions.

Parameter	Value
Sample Uptake Rate	0.04 mL/min
Nebulizer	MEINHARD® HEN
Spray Chamber	Asperon
Injector	2.0 mm id Quartz
RF Power	1600 W
Neb Gas Flow	0.36 L/min
Makeup Gas Flow	0.7 L/min
Platinum Isotope	195 amu
Dwell Time	50 μs
Sample Analysis Time	60 s

A2780/CP70 cells developed resistance by reducing cisplatin uptake in addition to up-regulating DNA repair².

We explored cisplatin uptake by performing a time course experiment to analyze how the distribution of cisplatin uptake changed over time within the cellular population. Both cell lines were treated for 1, 2, 4, and 8 hours with 30 μM cisplatin. During sample analysis, two real-time plots are produced: one shows the intensity response vs. sample number, while the second takes this data and converts it to a histogram showing the frequency of response vs. mass-per-cell; both plots are shown in Figure 4. After the data is collected, the mass distribution peak can be integrated, as shown in the third plot in Figure 4.

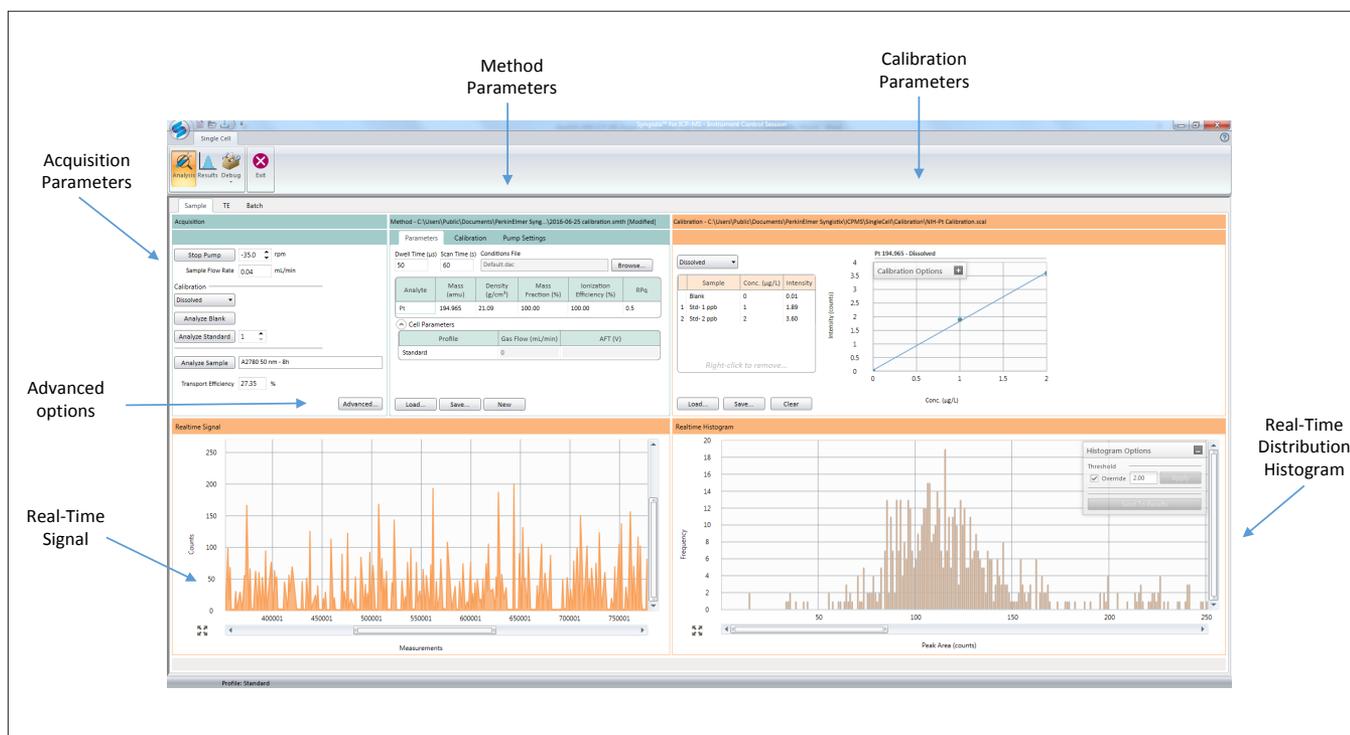


Figure 3. Syngistix Single Cell Application Software Module.

The cisplatin time course results are shown in Figure 5 for both cell lines. At one hour of cisplatin treatment, both cell lines show very little uptake of platinum. As time progresses, cells from both lines import increasing amounts of cisplatin and show a heterogeneous distribution. At eight hours post cisplatin treatment, the A2780 and A2780/CP70 cell lines have a subpopulation of cells with less cisplatin in comparison

to the rest of the cellular population. Additionally, there is a significant difference between the cell lines at eight hours as A2780 has an increased population of cells with more cisplatin than A2780/CP70. These results are concordant with what has been observed previously in the literature – A2780 cells accumulate more platinum than its cisplatin-resistant cell line A2780/CP70².

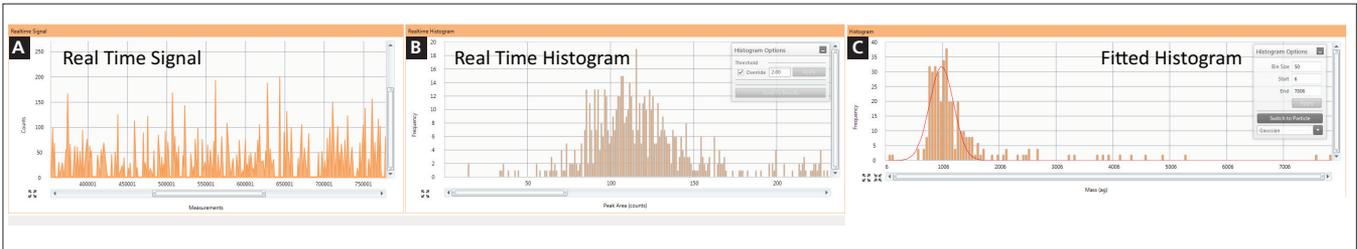


Figure 4. Real-time analysis of single cells exposed to cisplatin by SC-ICP-MS: (A) measurement vs. intensity; (B) peak area vs. frequency histogram; (C) integrated/fitted histogram.

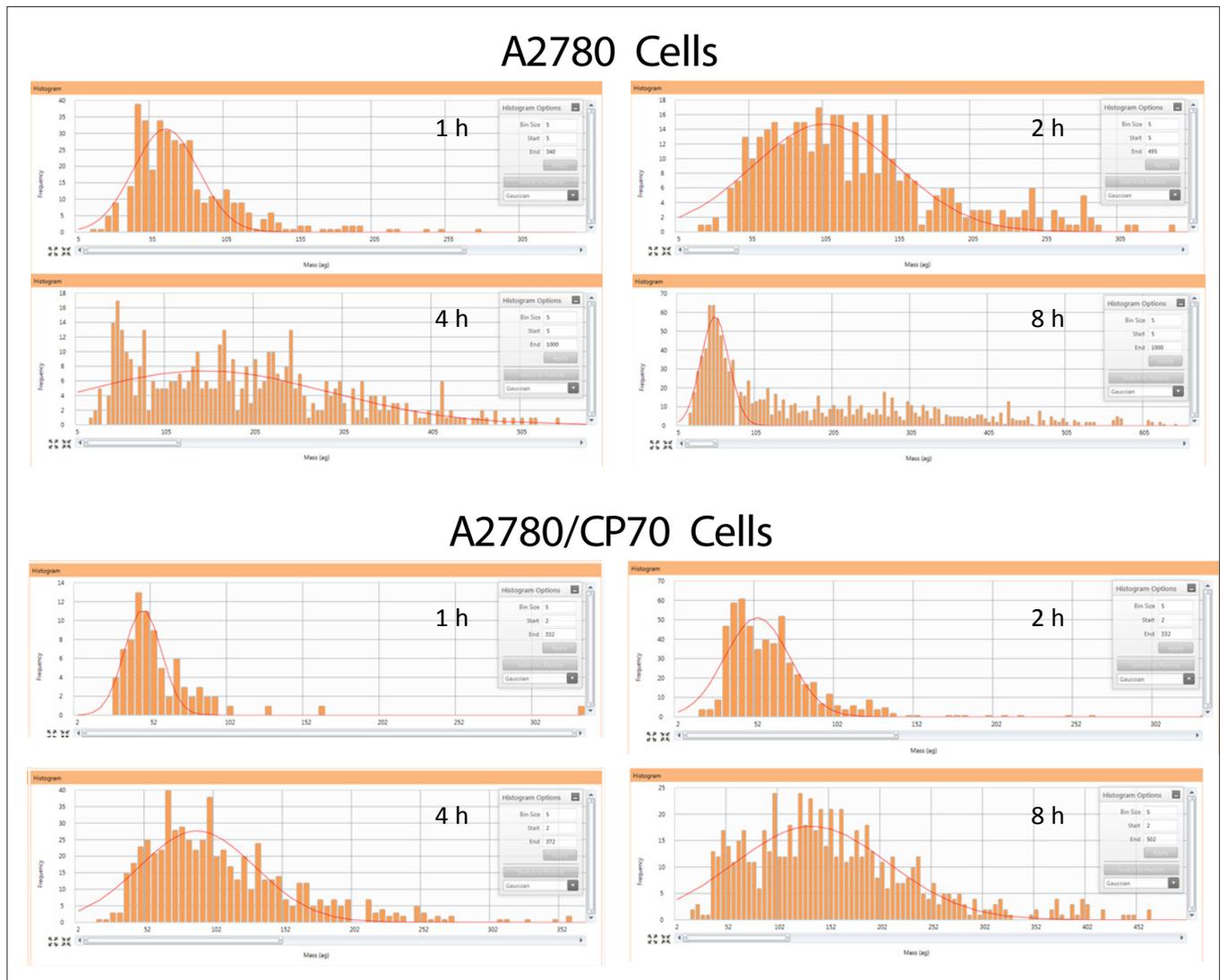


Figure 5. Cellular cisplatin uptake in A2780 and A2780/CP70 over time. Note: samples on different scales.

The time course data was summarized by fitting each histogram with a Log Normal curve. The mean intensity was determined for each time point and plotted to show the cisplatin uptake difference between A2780 and A2780/CP70 cells over time, as shown in Figure 6. Here, it is observed that cisplatin-sensitive A2780 cells have increased cisplatin uptake in comparison to the cisplatin-resistant A2780/CP70 cells over time.

To determine whether the heterogeneous distribution of cisplatin uptake was due to differences in the cell cycle, cells were serum starved. When cells are grown in tissue culture, the culture is typically a mixture of asynchronous cells growing at different rates, resulting in a variety of cells at different stages of the cell cycle, as represented in Figure 7. The four parts of the cell cycle are referred to as G1, S, G2, and M. The G1 phase is the first (and typically longest) growth phase, followed by the S phase, where DNA is synthesized and replicated in preparation for cell division. The G2 growth phase is next, followed by the M (or mitosis) phase where cells divide into two cells. Thus, it is plausible that the differences in cisplatin levels may be due to differences in the cell cycle phases. By starving the cells of serum, the growth factors are removed from the media and the cells will stall in the G1 phase.

The cisplatin uptake time experiments were repeated comparing the uptake difference between the serum-starved and control cells. Figure 8 shows the results of the serum starvation experiments where the mean intensity was determined for each time point and plotted. These results show that serum starvation had no effect on the uptake of cisplatin in either the A2780 or the A2780/CP70 cell lines; both show similar cisplatin uptake rates as the control cells. Thus, these results suggest that the heterogeneous distribution of cisplatin uptake in cells is not due to the cell cycle but rather some other unknown mechanism.

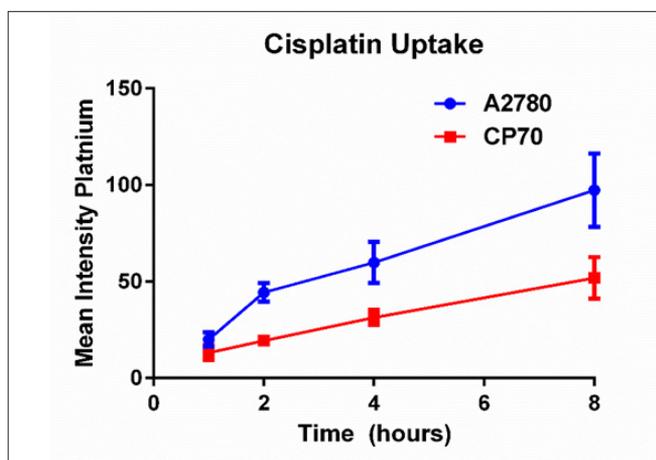


Figure 6. Summary of platinum mean intensity for each cell line over time. Results are a summary of n=3 independent experiments and plotted mean \pm standard deviation.

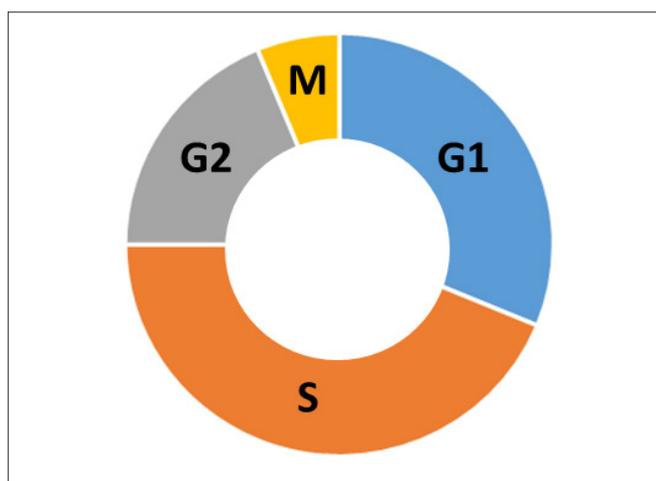


Figure 7. Phases of the cell cycle. G1 is the first growth phase. S is the synthesis phase. G2 is the second growth phase. M is the mitosis phase.

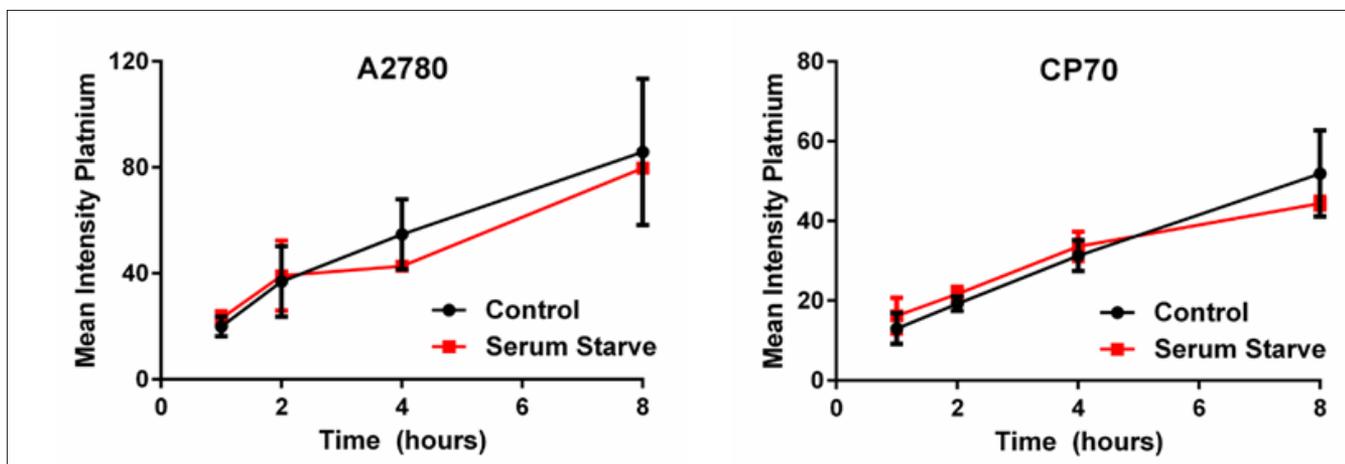


Figure 8. Plots of control vs. serum-starved cells for cisplatin uptake. These plots show that heterogeneous cellular cisplatin uptake is not due to cell cycle. Results are a summary of n=3 independent experiments and plotted mean \pm standard deviation.

Conclusions

Single Cell ICP-MS is a robust method for measuring platinum at the level of a single cell. Using the ovarian cancer cell lines A2780 and A2780/CP70, it was demonstrated that cisplatin uptake increases over time. The cisplatin uptake differs with the cisplatin resistant A2780/CP70 cell line, importing reduced levels in comparison to the A2780-sensitive cell line. The cellular variation in a population of cisplatin uptake was not due to differences in cell cycle, as serum-starving cells did not change the overall uptake of cisplatin. Intercellular variability of cisplatin uptake is due to other factors which have yet to be determined.

Single cell ICP-MS analysis allows for the quantitation of cisplatin within individual cells and is a better method for analyzing cisplatin uptake within a population of cells. Here, we demonstrate that internal cisplatin can be detected at the level of a single cell. The distribution of cisplatin uptake is heterogeneous in cells and differs from cell to cell, which probably more closely reflects what occurs within tumors.

Future studies using Single Cell ICP-MS will allow for the development of new strategies to increase cisplatin uptake in cells and novel pathways that influence cellular cisplatin import and export.

References

1. Amable L. (2016) Cisplatin resistance and opportunities for precision medicine. *Pharmacol Res.* 106:27-36.
2. Parker R.J. Eastman A. Bostick-Bruton F. Reed E. (1991) Acquired cisplatin resistance in human ovarian cancer cells is associated with enhanced repair of cisplatin-DNA lesions and reduced drug accumulation. *J Clin Invest.* 87(3):772-7.

Consumables Used

Component	Description	Part Number
Sample Uptake Tubing	Orange/red (0.38 mm id), PVC, flared, pack of 12	N0773111
Spray Chamber Drain Tubing	Grey/grey (1.30 mm id), Santoprene, pack of 12	N0777444
Platinum (Pt) Stock Standard	1000 ppm Pt, 125 mL	N9303791
60 nm Gold (Au) Nanoparticles	2.60E+10 particles/mL, 25 mL	N8142303
Sample Tubes	15 mL, case of 500 50 mL, case of 500	B0193233 B0193234

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