



## Effects of Platinum-based Chemotherapeutic Agents on ML-1 Thyroid Cancer Cells

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### Abstract

Recent literature demonstrates that platinum-based chemotherapeutic drugs in physiological solvents display higher efficacy in destabilizing cancer cells. As human cancer cells come in over 200 different varieties, it would be beneficial to test the efficacy of these drugs using a wider spectrum of cells. Utilizing the well-tested HeLa cervical cancer cells as a control for the effects of these drugs, we assessed the impact of platinum-based cisplatin, carboplatin, and oxaliplatin on ML-1 thyroid cancer cells. Through the XTT Viability assay, we found that ML-1 cells are more resistant to cisplatin and oxaliplatin with an  $IC_{50}$  value at least four times higher than those for the same drugs in HeLa cells. It has been consistently shown that the oxidative stress caused by these chemicals was more pronounced in HeLa cells than in ML-1 cells, but the only measurable results were found 24 hours after treatment. We also show that a high percentage of HeLa cells displayed apoptosis with even 20  $\mu$ M of these chemicals, which is directly comparable in effect to the 100  $\mu$ M of chemicals in ML-1 cells. Upon comparing the expression levels of pro-apoptotic enzymes in HeLa and ML-1 cells, we observed that when treated with 40  $\mu$ M of these chemicals, the levels of pro-apoptotic enzymes were statistically higher for HeLa cells than for ML-1 cells. Our research will provide new insight into the different capacities of each cell line and the treatment regimen for cancer patients in the future.

**Keywords:** ML-1; HeLa; Cisplatin; Cytotoxicity; Resistance

### Introduction

There were 1.9 million new cancer cases and more than 600,000 cancer deaths estimated for the year 2021 [1], and this trend would not change in 2022. With this steadily increasing annual expectation of new cancer cases, there comes a need for more chemotherapy options. Although platinum-based chemotherapeutic agents, Cisplatin, Carboplatin, and Oxaliplatin, are used quite often to treat different varieties of cancer [2,3], finding their common mechanisms is important in order to better understand their functions. These platinum-based chemicals all latch onto DNA and have the ability to cross-link DNA bases, which leads to an inhibition of DNA replication and promotes DNA damage [4].

Consequently, the extent of apoptosis in the affected cells is elevated [4-6].

Historically, scientists have used dimethylsulfoxide (DMSO) to solubilize many metal-based chemotherapeutics including cisplatin, carboplatin, and oxaliplatin due to the fact that they are highly soluble in DMSO up to 100 mM for cisplatin [7]. However, the structure of cisplatin is destabilized when solubilized in DMSO, which results in a low toxic effect [8,9]. Additionally, carboplatin and oxaliplatin are reactive, albeit to a lesser extent in DMSO, but their interactions result in mildly reduced cytotoxicity [10]. In contrast, it is widely known that cisplatin, carboplatin, and oxaliplatin have

lower solubility in water and physiological solvents like saline compared to DMSO, with their cytotoxic effect increased by a factor of at least 10 [11,12].

Hall, *et al.* and others have outlined these phenomena by showing that the  $IC_{50}$  values of chemicals like cisplatin can be decreased by approximately 45 times when solubilized in saline when compared to DMSO, suggesting that DMSO reduces the cytotoxic effect of cisplatin. In addition, when carboplatin and oxaliplatin were tested in a similar manner to cisplatin, it was found that the cytotoxic effects recorded were better for the non-DMSO solubilized chemicals [12].

Even with this, there is still a major gap in the number and types of cancer cells being researched compared to the total number of different cancer cells, along with other physiological buffers that have not yet been tested. The present study aims to more effectively accommodate these disparities by conducting cell viability and cytotoxicity experiments on the HeLa cervical cancer and ML-1 thyroid cancer cells, using the widely explored HeLa cells as a control for the ML-1 cells, which have never been utilized for this purpose. We present here that through the use of suggested physiological solvents, we found that ML-1 cells are more resistant towards the cytotoxic effects of these chemotherapeutic treatments, and we aim to discover the underlying mechanisms of this resistance, which we discuss in later sections.

## Materials and Methods

### Cell culture and cell viability assay

HeLa cervical cancer and ML-1 thyroid cancer cells were plated in a 96-well plate with a cell density of 7,000 for 24 hours. The cells were treated with three chemotherapeutic agents-Cisplatin, Oxaliplatin, or Carboplatin-in varying final concentrations of 0.165, 0.8, 4, 20, and 100  $\mu$ M through serial dilution for 48 hours. XTT, a second-generation tetrazolium dye, and PMS (N-methyl dibenzopyrazine methyl sulfate) solutions were mixed in a 200:1 ratio by volume. Each well was treated with 25  $\mu$ L of this suspension for 5 hours in a 37°C incubator. A spectrophotometer was used with settings to measure absorbance at 450 nm, and this value was subtracted by background absorbance measured at 630 nm to minimize unnecessary noise. The resulting net absorbance values were used to determine the average and standard errors by using Prism8 GraphPad. We utilized the one-way ANOVA test with the Dunnett

test setting, where a p-value lower than 0.05 indicated statistically significant differences. Prism 8 was also utilized to measure the  $IC_{50}$  values in  $\mu$ M of each chemical-treated cell.

### Oxidative stress measurement

HeLa and ML-1 cells were plated into an 18-well plate with a cell density of 10,000 cells per well for 24 hours with DMEM containing 10% FBS and antibiotics. Cisplatin, Oxaliplatin, or Carboplatin treated into the wells in concentrations of 0.165, 0.8, 4, 20, and 100  $\mu$ M. They were then placed into an incubator for varying amounts of time, specifically 4, 24, and 48 hours. After these incubation times, we treated each well with 5  $\mu$ M of CellROX green (Invitrogen) and incubated again for 30 minutes at 37°C. Afterward, each well was washed 3 times with 1X PBS and visualized utilizing a spinning confocal microscope with a 10x objective lens and exposure time of 100 milliseconds. The excitation/emission filter was set at 488 and 530 nm, respectively. All recorded images were exported into ImageJ to determine the average and standard deviations of fluorescent intensities for each treatment by using 20 cells in triplicate. Utilizing Prism8 GraphPad, we used a one-way ANOVA test with the Dunnett test setting to determine any significant differences between groups. A p-value lower than 0.05 was considered statistically different from the non-treated control.

### Apoptosis measurement

HeLa and ML-1 cells were plated into a 24-well plate with a cell density of 70,000 cells per well for 24 hours. We treated each well with 20 or 40  $\mu$ M of Cisplatin, Oxaliplatin, or Carboplatin at two different times (24 and 48 hours, respectively). We also treated ML-1 cells with 100  $\mu$ M of these chemicals for 48 hours only. Cells were harvested using Trypsin without EDTA solution, after which the harvested cells were centrifuged at 1000g for 10 minutes. The collected pellet of cells was resuspended with 1X Annexin V Binding Buffer, with Propidium iodide (PI) and Annexin V-APC being applied as well. After 30 minutes of incubation in the dark, the cells were analyzed utilizing an Attune NxT Flow Cytometer with the BL-2 (Excitation/Emission setting of 650/660 nm) and RL-1 (Excitation/Emission setting of 535/617 nm) channels for measuring the Annexin V-APC and PI, respectively. The percentage of cells in Early Apoptosis and Late Apoptosis were determined utilizing the Attune NxT software. Using Prism8 GraphPad, the statistical differences between the average percentages of Apoptosis induced by the chemotherapeutic agents and that of the NTC were determi-

ned. For this, we used one-way ANOVA tests with the Dunnett test setting, and p-values lower than 0.05 were considered statistically different.

### Proapoptotic protein expression measurement

HeLa and ML-1 cells were plated in a 6-well plate with a cell density of 200,000 cells per well for 24 hours. They were treated with 40  $\mu\text{M}$  of the chemotherapeutic agents for 24 or 48 hours. The cells were lysed utilizing 100  $\mu\text{L}$  of RIPA buffer, and the resulting cell lysate was subjected to the Bradford Assay in order to measure the protein concentration. A total of 14  $\mu\text{g}$  of protein from each cell lysate was applied to SDS gel electrophoresis for 60 minutes at 120 volts. The separated proteins were transferred to a nitrocellulose membrane at 4°C for an hour at 350 mA. Then, the blot was incubated with TBST (Tris-buffered Saline with 0.1% tween 20) containing 5% non-fat dry milk overnight at 4°C. The blot was then incubated with the following primary antibodies from Cell Signaling Technology: Catalase (D5N7V) Rabbit mAb, Bax (D3R2M) Rabbit mAb, and Caspase-3 (D3R6Y) Rabbit mAb. In addition to those, mouse monoclonal anti-GAPDH antibody (G-9) was incubated for 24 hours at 4°C, as were all of the primary antibodies from Cell Signaling Technology. The blots were washed three times TBST for 10 minutes each wash. The secondary antibodies conjugated with Horse Radish peroxidase (HRP) were incubated with the blots for an hour prior to the washing steps. Afterward, the blots were treated with Radiance Q as a chemiluminescent substrate to develop chemiluminescence in the target protein bands, which was detected by an Azure biosystems gel imaging workstation. Utilizing ImageJ, the band intensity of each protein was divided by the band intensity of the corresponding GAPDH in each lane in a triplicate manner to determine the averages and standard deviations. These values and any statistical differences were found with Prism8 GraphPad by using the one-way ANOVA tests with the Dunnett test setting. P-values lower than 0.05 were considered statistically different.

## Results

### ML-1 thyroid cancer cells are more resistant to the platinum-based chemotherapeutic agents

A group of researchers recently reported that chemotherapeutic agents which are solubilized in DMSO are less effective than when they solubilize in physiological solutions. Although they ob-

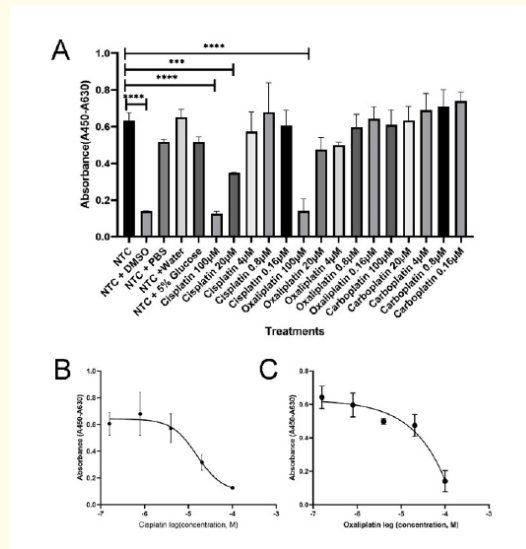
served these results in a number of different cancer cell lines, there is still a necessity for the research to be expanded to more cancer cell lines in order to provide more insight into the efficacy of widely used chemotherapeutic agents such as Cisplatin, Oxaliplatin, and Carboplatin. In the present research, we utilized the HeLa and ML-1 cancer cell lines. These cells were treated with Cisplatin solubilized in 1x PBS, Oxaliplatin in Water, or Carboplatin in 5% glucose for 48 hours.

There was a reduction in cell viability of HeLa cells in response to the treatment of 20 and 100  $\mu\text{M}$  of Cisplatin, resulting in an  $\text{IC}_{50}$  value of 16  $\mu\text{M}$  (Figure 1 A&B). For Oxaliplatin, only 100  $\mu\text{M}$  showed any noticeable viability defects in the cells (Figure 1C), and the calculated  $\text{IC}_{50}$  value for it was 40.28  $\mu\text{M}$ . In Carboplatin, there was no reduction in cell viability in any of the concentrations. In contrast, ML-1 cells showed lesser levels of reduction in cell viability overall (Figure 2A), even with the same concentrations of chemicals treated. The  $\text{IC}_{50}$  value of Cisplatin in ML-1 was 67.59  $\mu\text{M}$ , while Oxaliplatin's was 85  $\mu\text{M}$  (Figure 2 B&C). This indicates that HeLa cells are more sensitive, or ML-1 cells are more resistant to these chemical treatments. We recently reported that Cisplatin solubilized in DMSO resulted in an  $\text{IC}_{50}$  value of 500  $\mu\text{M}$  in ML-1 cells [13,14]. Therefore, platinum-containing chemotherapeutic agents solubilized in DMSO are comparatively less effective than the same agents in physiological salt solutions, which is consistent with previous findings [12].

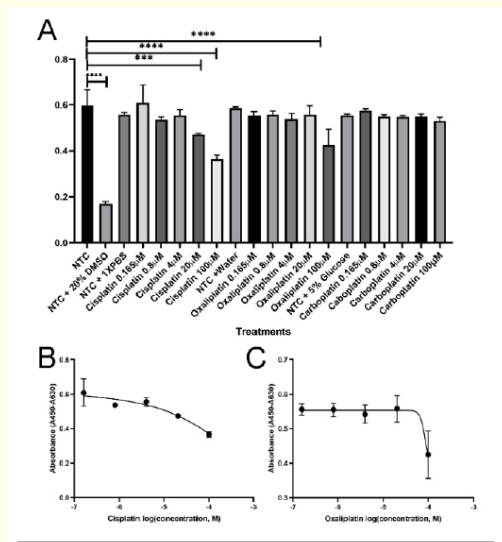
### Measurement of reactive oxygen species

Based on our XTT assay's results, Cisplatin and Oxaliplatin caused a significant reduction of cell viability with 20 and 100  $\mu\text{M}$  concentrations in HeLa cells (Figure 1). However, for ML-1 cells, only 100  $\mu\text{M}$  of these chemicals induced cell viability defects (Figure 2). With these results, we hypothesized that these chemotherapeutic agents are likely inducing oxidative stress by contributing to the production of more Reactive Oxygen Species (ROS), which might be a factor for the reduced cell viability. In order to measure the amount of oxidative stress induced by these chemicals, we treated both cells with 5 $\mu\text{M}$  of CellROX green and analyzed the fluorescent intensities of each treated sample.

The group of samples with HeLa cells treated with the chemotherapeutic agents for 4 hours displayed essentially no differences in ROS production compared to that of the NTC (Figure 3 A-C).



**Figure 1:** XTT cell viability assay to determine the toxicity that concentrations of 0.165, 0.8, 4, 20, 100 µM of Cisplatin, Oxaliplatin, or Carboplatin may cause to HeLa. (A) The absorbance of each graph measured at A450-A630 nanometers is compared between each concentration and the non-treated control for each chemical, which indicates any statistically significant differences in HeLa cell viability. (B) The IC<sub>50</sub> in µM was determined for the Cisplatin treated cells. The x-axis represents the concentration of Cisplatin in log form. (C) Determination of IC<sub>50</sub> value for Oxaliplatin. Statistical significance is indicated by the asterisks; \* indicates a p-value<0.05, \*\* indicates <0.01, \*\*\* indicates<0.001, and \*\*\*\* indicates<0.0001.

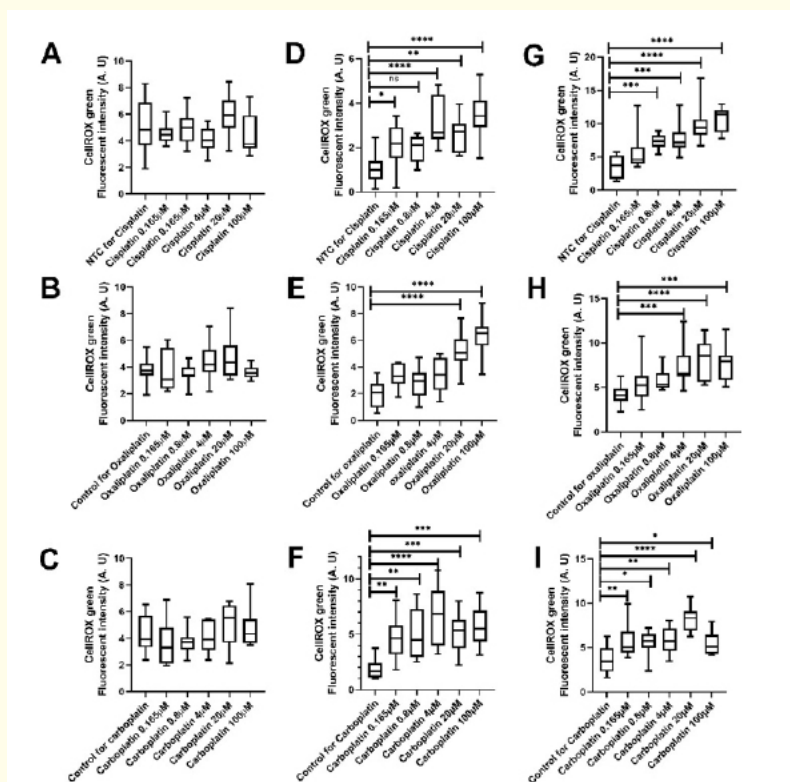


**Figure 2:** Measurement of cell viability in ML-1 Thyroid cancer cells. (A) Varying concentrations of Cisplatin, Oxaliplatin, and Carboplatin were treated for 48 hours as stated in the Methods section for XTT assay. Statistical significance is indicated by the asterisks; \* indicates a p-value<0.05, \*\* indicates <0.01, \*\*\* indicates<0.001, and \*\*\*\* indicates<0.0001. (B) The IC<sub>50</sub> was determined for the Cisplatin- treated cells. The x-axis represents the concentration of Cisplatin utilized in log form. (C) The IC<sub>50</sub> for the Oxaliplatin-treated cells was determined. The x-axis also represents the concentration in log form.

However, at 24 and 48 hours of treatment, the ROS levels of several concentrations of these three chemotherapeutic agents showed significantly elevated levels of ROS production when compared to the NTC (Figure 3 D-I). Therefore, we came to the conclusion that the viability defects in HeLa cells we observed in the XTT Assay were at least partially due to the elevated production of ROS. In the ML-1 samples, both 4- and 24-hour data showed no noticeable induction of ROS except for 100  $\mu$ M cisplatin-treated samples (Figure

4 A-F). However, at 48 hours, the higher concentrations of 20 and 100  $\mu$ M in cisplatin and oxaliplatin treated samples revealed statistically significant elevations of ROS as opposed to no elevation of ROS in any carboplatin-treated samples (Figure 4 G-I).

Altogether, these results indicate that HeLa cells are more prone to the production of ROS when treated with these chemicals when compared with ML-1 cells treated under the same conditions.



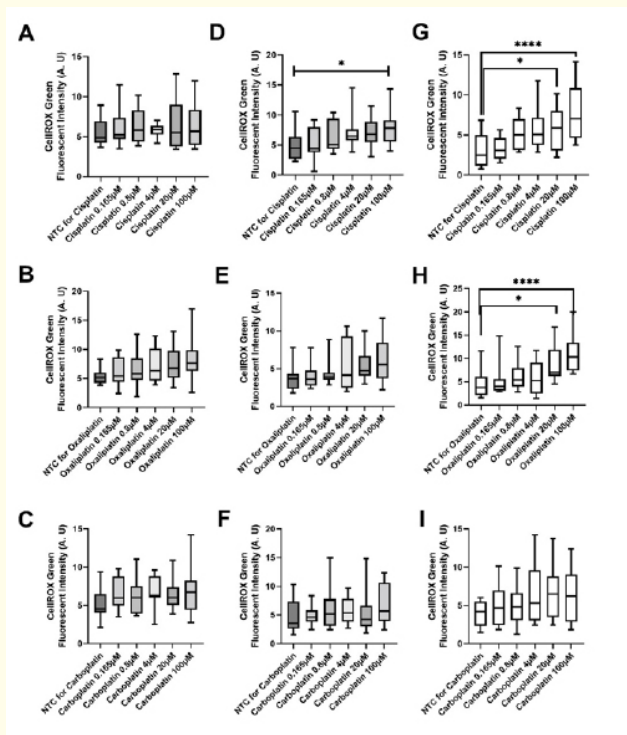
**Figure 3:** Measurement of levels of Reactive Oxygen Species (ROS) caused in HeLa cells by different concentrations of Cisplatin, Oxaliplatin, and Carboplatin. (A, D, G) Starting from the far left, each graph in the first row represents the different times at which the ROS was measured (4, 24, and 48 hours) in Cisplatin-treated cells. (B, E, H) ROS measurement for Oxaliplatin treated cells at 4, 24, and 48 hours (from left to right in the second row). (C, F, I) Measurement of ROS for Carboplatin treated cells at 4, 24, and 48 hours (from left to right in the third row). Statistical significance is indicated by the asterisks; \* indicates a p-value<0.05, \*\* indicates <0.01, \*\*\* indicates<0.001, and \*\*\*\* indicates<0.0001.

**Apoptosis induction measurement**

The IC<sub>50</sub> value of Cisplatin-treated HeLa cells was 16  $\mu$ M. Therefore, we decided to treat HeLa cells with 20  $\mu$ M of all the chemo-

therapeutic agents for 24 hours. Interestingly, we found that the 24- hour incubation was not sufficient to induce either Early or Late Apoptosis (Figure 5 A&B). We then incubated a new batch of



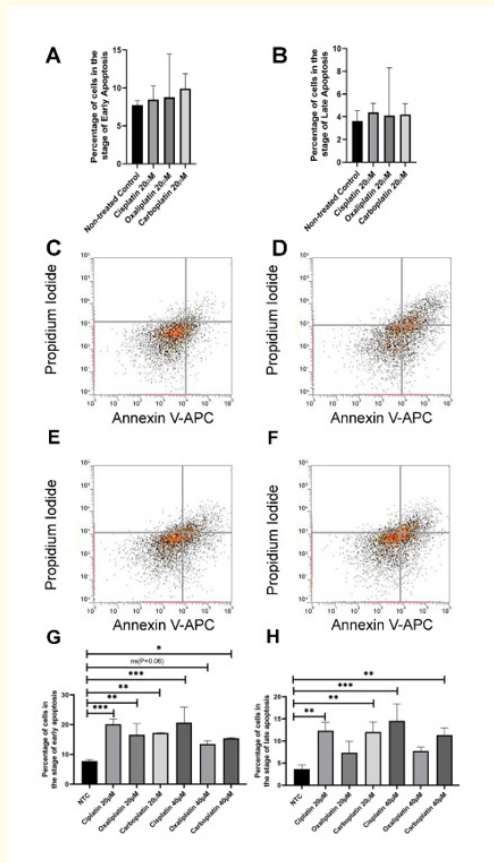


**Figure 4:** Measurement of oxidative caused in ML-1 cells by different concentrations of Cisplatin, Oxaliplatin, and Carboplatin. (A, D, G) Starting from the far left, each graph in the first row represents the different times at which the ROS was measured (4, 24, and 48 hours). (B, E, H) ROS measurement for Oxaliplatin treated cells at 4, 24, and 48 hours (from left to right in the second row). (C, F, I) Measurement of ROS for Carboplatin treated cells at 4, 24, and 48 hours (from left to right in the third row). Statistical significance is indicated by the asterisks; \* indicates a p-value<0.05, \*\* indicates <0.01, \*\*\* indicates<0.001, and \*\*\*\* indicates<0.0001.

treated cells for 48 hours with two different concentrations, including the previously used 20 μM (Figure 5 C-G) as well as 40 μM of each chemical (Figure 5H). At 48 hours, the 20 μM treated cells all displayed statistically significant differences in the amount of early and late apoptosis, but Oxaliplatin treated cells did not show this difference in late apoptosis for 20 and 40 μM treated cells (Figure 5 G&H). Through this analysis, we determined that the trend for these treatments is that 20 μM is a sufficient concentration to induce noticeable apoptosis within 48 hours in HeLa cells.

This suggests that the cytotoxicity observed by the XTT assay is partially attributed to cell apoptosis induced by these chemicals. However, it is likely that the elevated ROS production might directly affect the elevated levels of apoptosis in the cells.

In ML-1 cells, both 20 and 40 μM of chemical treatments had no effect on the levels of apoptosis shown after 24 hours (Figure 6 A&B). At 48 hours, the 20 and 40 μM cisplatin-treated cells demonstrated marginal increases in early apoptosis, but the average difference was not especially significant compared to differences found between the NTC and treated cells in the HeLa apoptosis measurements at 48 hours (Figure 6C, Figure 5G). It was only after 100 μM of these chemicals where significant induction of both early and late apoptosis was detected (Figure 6 E&F). Together, HeLa cells are more easily provoked to induce apoptosis at similar concentrations of chemical treatments when compared to ML-1 cells, even at lower concentrations like 20 and 40 μM.

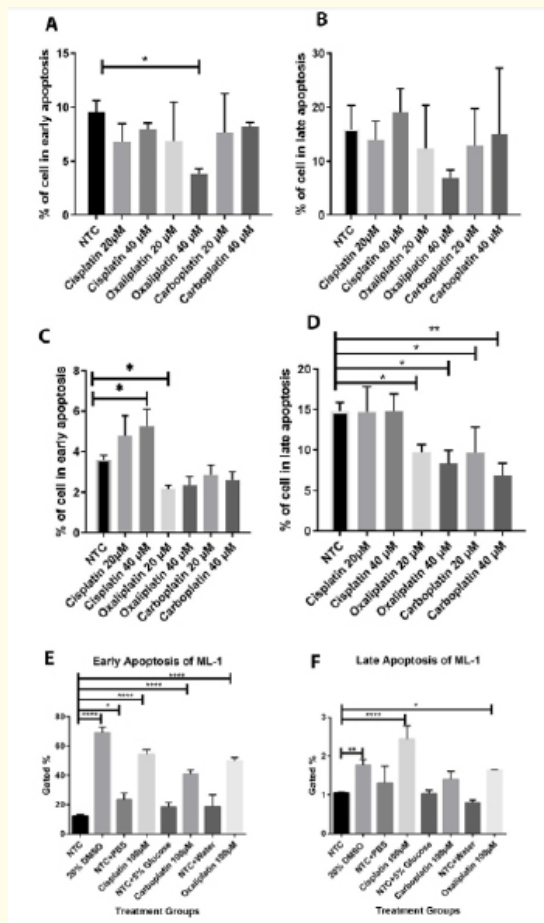


**Figure 5:** Analysis of apoptosis in HeLa cells in response to the treatment of Cisplatin, Oxaliplatin, and Carboplatin in varying concentrations and treatment durations. (A) Determination of the levels of early apoptosis in cells with 24-hour treatment of 20 μM Cisplatin, Oxaliplatin, or Carboplatin. (B) Percentage of cells in late apoptosis after 24 hours of treatment with 20 μM of these chemicals. (C-F) Representative flow cytometry plots of apoptosis measurements at 48 hours of treatment; (C) Non- treated control, (D) 20 μM Cisplatin, (E) 20 μM Oxaliplatin, and (F) 20 μM Carboplatin. Propidium Iodide and Annexin V-APC were utilized to label cells for the identification of early or late-stage apoptosis. (G) Quantification of the percentage of cells in early apoptosis 48 hours after treatment with 20 or 40 μM of chemicals. (H) Percentage of cells in late apoptosis 48 hours after treatment with 20 or 40 μM of chemicals. Statistical significance is indicated by the asterisks; \* indicates a p-value<0.05, \*\* indicates<0.01, \*\*\* indicates<0.001, and \*\*\*\* indicates<0.0001.

**Proapoptotic protein detection via western blot analysis**

After observing the results of the XTT assay, ROS measurement, and Apoptosis measurement, we decided to measure the expression of three different proteins that are known to become highly expressed in response to apoptotic signals [15,16]. As such, HeLa cells incubated with the three chemotherapeutic agents for 24 hours were subjected to a Western Blot (Figure 7). After 24 hours

of incubation with these chemicals, including cisplatin and oxaliplatin, a major fraction of cells undergo apoptosis. Therefore, the cells were incubated with these chemicals for 24 hours. The 40 μM cisplatin- treated cells showed an elevation in the average expression of Bax and Caspase-3 (Figure 7 A&C), but the change was not statistically significant in catalase expression. There was also an increase in the average expression of Bax after the 40 μM treatment



**Figure 6:** Apoptosis measurement in ML-1 cells (A-B) Early and late apoptosis at 24-hour incubation with three different chemotherapeutic agents. (C-D) Early and late apoptosis at 48-hour incubation with the chemicals. (E-F) Percentage of cells in early and late apoptosis 48 hours after treatment of 100 μM of chemicals. Statistical significance is indicated by the asterisks; \* indicates a p-value < 0.05, \*\* indicates < 0.01, \*\*\* indicates < 0.001, and \*\*\*\* indicates < 0.0001.

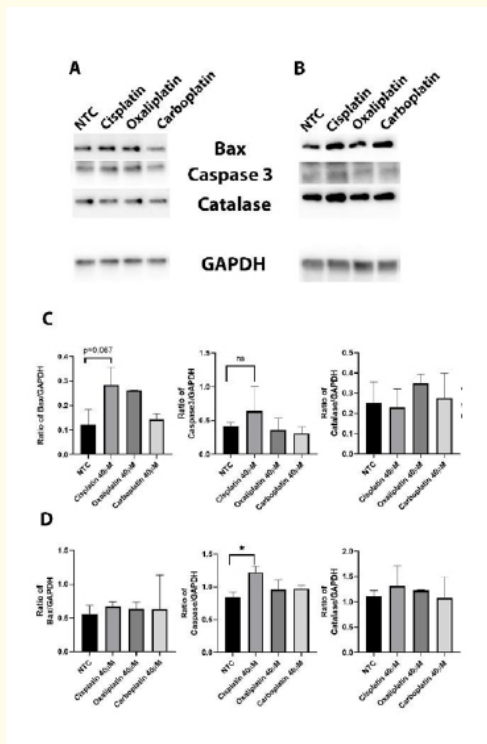
of oxaliplatin similar to that of the cisplatin-treated result (Figure 7 A&C), but it was not statistically significant. Overall, Carboplatin-treated cells showed no discernable difference in expression of all three proteins when compared to the NTC upon treatment with these chemicals (Figure 7 A&C).

In ML-1, the expression of all proteins with any treatment of the chemicals showed no difference when compared to the NTC except for 40 μM of cisplatin with caspase3 expression having a statistically significant increase (Figure 7 B&D).

### Discussion

DMSO is a very well-known universal solvent for anti-cancer drugs containing platinum. However, it is recommended that physiological solutions such as saline, PBS that contains chloride, water, and water with 5% glucose are used to solubilize these chemicals instead of DMSO due to the findings of at least two recent reports, which demonstrated that DMSO destabilizes platinum-containing chemotherapeutic agents including cisplatin, oxaliplatin, and carboplatin [11,12]. With this train of thought, Davis, *et al.* further





**Figure 7:** Measurement of the level of expression for Caspase-3, BAX, and Catalase in HeLa cells 24 hours after treatment of 40  $\mu$ M Cisplatin, Oxaliplatin, or Carboplatin. Cell Lysates were made after cell incubation with RIPA buffer. After quantification of protein amount in each cell lysate via the Bradford Assay method, 14  $\mu$ g of protein in each lane were applied to SDS gel electrophoresis, followed by wet protein transfer. The indicated antibodies and HRP-conjugated antibodies were sequentially treated with nitrocellulose membrane. Chemiluminescence resulting from the treatment of HRP substrate was recorded as stated in the Methods section. (A) Western blot results with HeLa cells. (B) Western blot results with ML-1 cells. (C) The ratio of each target protein band intensity to GAPDH intensity is indicated on the Y-axis.

supported the notion that DMSO decreases the cytotoxic effect of anticancer ruthenium complex KP1019 [18], which is a metal-based chemotherapeutic agent similar to the platinum-based chemicals we used in the present study. One possible explanation for the reduced cytotoxicity upon treatment of chemicals with DMSO was investigated by Tanley, *et al.* where they found that treatment of cisplatin and carboplatin with DMSO caused the chemotherapeutic agents to bind to hen egg-white lysozyme (HEWL) rather than binding to DNA to distort it [19]. The present study is thus an expansion of this trend of research by delving into the effects these che-

micals may have on human cancer cells in addition to the already existing data by Hall, *et al.* in 2014, providing additional yet novel insight in regards to these chemicals and different types of cancers they are used to treat.

**Difference in IC<sub>50</sub> values with different cells**

It was nicely shown through the use of HeLa and DLD-1 colorectal adenocarcinoma cancer cells that the platinum-based chemotherapeutic agents in non-DMSO solvents have remarkably increased cytotoxicity in comparison to when the chemicals are treated

with DMSO [12]. We also used HeLa cells as a benchmark in order to branch out with types of cancer cells that have not been researched in this capacity previously; our choice of new cancer cell was ML-1 thyroid cancer. Interestingly, the  $IC_{50}$  values for HeLa cells that were found by Hall, *et al.* are lower than our due to a difference in experimental conditions. For instance, they chose to treat the cells for 72 hours whereas we treated our cells for 48 hours maximum. Another difference was in the solvents being used, as they utilized saline both with and without mannitol for the treatment of cisplatin. For carboplatin and oxaliplatin, they utilized water with and without 5% glucose. In our research, we solubilized cisplatin in 1X PBS, oxaliplatin in water, and carboplatin in water with 5% glucose. Thus, we can conclude that the effects of these platinum-based chemotherapeutic agents can vastly vary in  $IC_{50}$  values based on what types of cells are used, the solvents used, and the experimental duration and conditions used. However, it is still clear that ML-1 cells in our experiment are more resistant than HeLa cells against the chemicals.

### Why is HeLa more sensitive than ML-1?

One study has shown that cancer patients react differently to standardized treatments due to the fact that each individual's genetic and physical characteristics differ vastly from one another [20,21]. Likewise, on the fundamental level of individual cells, each can react to these treatments in a unique manner. For instance, when treated with docetaxel dissolved in DMSO, four different cancer cells lines MCF-7 (breast cancer), SKOV-3 (ovarian cancer), PC-3 (prostate cancer), and NCI-H2126 (non-small cell lung cancer) had  $IC_{50}$  values of 5, 83.7, 6.4, and 5  $\mu\text{mol/mL}$ , respectively [22]. Along the same lines, we reported that HeLa and ML-1 showed different  $IC_{50}$  levels in response to the platinum-based chemotherapeutic agents (Figure 1&2). It appears that in order to find the most efficient and clinically effective treatment for any individual cancer cell will inevitably lead to cell-based toxicity assays being needed to determine optimal treatments, as Florento., *et al.* and our research has shown. Yet, the fundamental mechanisms behind this difference in reaction between cell lines are poorly understood at this moment. While we cannot answer everything about why these cells react differently, we are at least able to propose the base regarding the differences between HeLa and ML-1. The consistency found between our ROS and apoptosis data points to more resistance in ML-1 cells, which we can connect to a lower level of BAX expression in ML-1 cells relative to HeLa cells. Although there are

many possible ways to gain resistance to chemotherapeutic agents, we can propose the possibility that many unknown factors along with MDR1 elevation may contribute to the higher resistance found in ML-1.

### Limitations of this research

One of the main limitations of this research is that we were only able to use two different types of cancer cell lines, which are HeLa and ML-1. A great way to branch out in the future would be to utilize more kinds of cancer cells in order to make the knowledge of how they are affected by chemotherapeutic agents more standardized. Having a broader array of chemotherapeutic agents would also be one way to expand this research. Knowing how many kinds of cancer cells react to many different chemicals would surely increase the breadth of our knowledge in regards to what cancer should be treated with which therapy. Along with different cell lines, having different solvents used in the delivery of the chemicals would be very eye-opening. As evidenced by the research from Hall, *et al.* stating that DMSO reduces the toxicity of platinum-based chemotherapeutic agents, it is important to know how a variety of different solvents can affect the efficacy of different chemicals. One more important thing that we would like to pursue in the future regarding the research is to measure the amount of platinum secreted from the treated cells in relation to the amount of MDR1 expressed.

### Conclusions

All of the results from the performed experiments and assays point toward an elevation in oxidative stress and apoptosis in response to the treatment of Cisplatin, Oxaliplatin, and Carboplatin, with Cisplatin showing the most severe effects on HeLa cells rather than on ML-1 cells, even with just 20  $\mu\text{M}$  concentrations, which is about the  $IC_{50}$  value of Cisplatin. Although there was no cytotoxicity measured for 20  $\mu\text{M}$  of Oxaliplatin and Carboplatin by the XTT assay, the ROS measurement and Apoptosis experiment data revealed that these concentrations were sufficient to induce elevated oxidative stress and apoptosis. This indicates that the XTT assay must be coupled with the ROS and Apoptosis experiments in order to fully understand the amount of chemicals needed to induce cell toxicity. Finally, we propose that the higher expression of BAX in HeLa cells can be attributed to the higher extent of sensitivity that they demonstrate in comparison to ML-1 cells.

### Supplementary Materials

N/A.

## Author Contributions

Daniel Kim conducted all experiments shown in figures 1, 3, 5, and 7. He also wrote the whole draft of this manuscript. Min Zhang and Nhi Le conducted all experiments in figures 2, 4, 6, and 7. Seth Harris conducted experiments in figure 7 as well. Dr. Kyoungtae Kim designed all experiments and provided guidance for all research and analysis of data, along with the editing of the manuscript.

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## Data Availability Statement

N/A.

## Acknowledgments

N/A.

## Conflicts of Interest

The authors declare no conflict of interest.

## Bibliography

1. Cancer-Facts-and-Figures-2021.Pdf, American Cancer Society (2021).
2. S Schoch., *et al.* "Comparative Study of the Mode of Action of Clinically Approved Platinum-Based Chemotherapeutics". *International Journal of Molecular Sciences* 21.18 (2020).
3. JP Becker., *et al.* "Cisplatin, oxaliplatin, and carboplatin unequally inhibit in vitro mRNA translation". *Toxicology Letter* 225.1 (2014) 43-47.
4. S Dasari and PB Tchounwou. "Cisplatin in cancer therapy: molecular mechanisms of action". *European Journal of Pharmacology* 740 (2014): 364-378.
5. RK Mehmood. "Review of Cisplatin and oxaliplatin in current immunogenic and monoclonal antibody treatments". *Oncology Review* 8.2 (2014): 256.
6. GF De Sousa., *et al.* "Carboplatin: molecular mechanisms of action associated with chemoresistance". *Brazilian Journal of Pharmaceutical* 50.4 (2014).
7. DA Pereira and JA Williams. "Origin and evolution of high throughput screening". *British Journal of Pharmacology* 152.1 (2007): 53-61.
8. SJ Kerrison and PJ Sadler. "Solvolysis of Cis-Pt (NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>. in Dimethyl Sulphoxide and Reactions of Glycine with PtCl<sub>3</sub> (Me<sub>2</sub>SO).- as Probed by <sup>195</sup>Pt Nuclear Magnetic Resonance Shifts and <sup>195</sup>Pt-<sup>15</sup>N Coupling Constants". *Journal of the Chemical Society, Chemical Communications* 23 (1977).
9. N Farrell., *et al.* "Chemical Properties and Antitumor Activity of Complexes of Platinum Containing Substituted Sulfoxides PtCl (R'R''SO) (Diamine).NO<sub>3</sub>. Chirality and Leaving-Group Ability of Sulfoxide Affecting Biological Activity". *Inorganic Chemistry* 29.3 (1990).
10. YW Yi and I Bae. "Effects of solvents on in vitro potencies of platinum compounds". *DNA Repair (Amst)* 10.11 (2011): 1084-1085.
11. HP Varbanov., *et al.* "Oxaliplatin reacts with DMSO only in the presence of water". *Dalton Transactions* 46.28 (2017): 8929-8932.
12. MD Hall., *et al.* "Say no to DMSO: dimethylsulfoxide inactivates cisplatin, carboplatin, and other platinum complexes". *Cancer Research* 74.14 (2014): 3913-3922.
13. H Rizvanovic., *et al.* "Chlorotoxin Conjugated with Saporin Reduces Viability of ML-1 Thyroid Cancer Cells in Vitro". *BioRxiv* (2019).
14. H Rizvanovic and K Kim. "Novel Cyanoximate Pt (DECO)<sub>2</sub> as an Anti-Cancer Drug Using ML1 Thyroid Cancer Cells". *Acta Scientific Microbiology* 4.6 (2021).
15. D Ali., *et al.* "ROS- dependent Bax/Bcl2 and caspase 3 pathway-mediated apoptosis induced by zineb in human keratinocyte cells". *OncoTargets and Therapy* 11 (2018): 489-497.
16. G Valdameri., *et al.* "Involvement of catalase in the apoptotic mechanism induced by apigenin in HepG2 human hepatoma cells". *Chemico-Biological Interactions* 193.2 (2011): 180-189.
17. A Haque., *et al.* "MDR1 Gene Polymorphisms and Its Association With Expression as a Clinical Relevance in Terms of Response to Chemotherapy and Prognosis in Ovarian Cancer". *Frontiers in Genetics* 11 (2020): 516.

18. J Davis., *et al.* "DMSO reduces the cytotoxicity of anticancer ruthenium complex KP1019 in yeast". *MicroPublication Biology* 2021 (2021).
19. SW Tanley., *et al.* "Structural studies of the effect that dimethyl sulfoxide (DMSO) has on cisplatin and carboplatin binding to histidine in a protein". *Acta Crystallographica Section D* 68 (Pt 5) (2012): 601-612.
20. MR Spitz., *et al.* "Integrative epidemiology: from risk assessment to outcome prediction". *Journal of Clinical Oncology* 23.2 (2005): 267-275.
21. RS Huang., *et al.* "Effect of population and gender on chemotherapeutic agent-induced cytotoxicity". *Molecular Cancer Therapeutics* 6.1 (2007): 31-36.
22. L Florento., *et al.* "Comparison of Cytotoxic Activity of Anticancer Drugs against Various Human Tumor Cell Lines Using In Vitro Cell-Based Approach". *International Journal of Biomedical Science* 8.1 (2012): 76-80.