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Toxicity of Platinum Containing Compounds with Variable Leaving Ligands on Cancer Cells

Brooke Duke

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TOXICITY OF PLATINUM CONTAINING COMPOUNDS WITH VARIABLE LEAVING LIGANDS ON CANCER CELLS

A Capstone Experience/Thesis Project
Presented in Partial Fulfillment of the Requirements for
the Degree Bachelor of Science with
Honors College Graduate Distinction at Western Kentucky University

By
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*****

Western Kentucky University
2016

CE/T Committee: Approved by
Professor Kevin Williams, Advisor
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Advisor
Department of Chemistry
ABSTRACT

Currently there are three platinum anticancer drugs on the market: cisplatin, oxaliplatin, and carboplatin. These compounds have variable leaving ligands, which are the locations on compounds that will be replaced by biological targets (DNA and protein). The purpose of this research is testing platinum compounds that have a nonleaving ethylenediamine or diaminocyclohexane ligand, similar to the nonleaving ligands found in cisplatin and oxaliplatin, and variable leaving ligands. Three compounds were tested including oxaliplatin, ethylenediamine(oxalato)platinum(II) (Pt(en)(ox)), and dichloro(ethylenediamine)platinum(II) (Pt(en)Cl₂). MTT assays were performed on cancer cells treated with the platinum compounds for twenty-four hours. In MTT assays, the living cells can reduce a tetrazolium salt to a purple formazan that is quantified to determine percent cell survival. The testicular cancer cell line NTERA-2 showed a higher sensitivity to Pt(en)(ox) than the colorectal cancer cell line HT-29. The NTERA-2 cells were 2.5 times more sensitive to Pt(en)(ox) than to the therapeutic standard oxaliplatin. Also, the HT-29 and NTERA-2 cancer cell lines had similar toxicities to oxaliplatin.

Keywords: Cisplatin, Oxaliplatin, Platinum, Anticancer
Dedicated to my friends and family
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Finally, I would like to thank my friends and family who have supported me throughout my college experience.
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Major Field: Chemistry
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CHAPTER 1

INTRODUCTION

Michele Peyrone first synthesized \textit{cis}-diamminedichloroplatinum(II), which is now known as cisplatin, in 1844 [1]. Cisplatin would later become a widely used cancer treatment and lead to the development of a new family of anticancer treatments. The Rosenberg laboratory was the first to discover that platinum-containing compounds interfered with cell growth. In the 1960s, the Rosenberg laboratory unintentionally discovered the anticancer effects of platinum after observing a halt in cell division of \textit{Escherichia coli} bacteria when placed into solution with platinum electrodes [2]. Originally it was thought that the halt in cell division was due to the electric field caused by the electrodes, but it was later discovered that the platinum in the electrodes was responsible for the halt in cell division. Further studies with a sarcoma mouse model showed that the platinum compound cisplatin had the ability to kill cancer cells and shrink tumors [2]. However, there was concern over the toxic effects of heavy metals on the body. It was later shown that in low doses the mice still showed tumor shrinkage and less toxic effects [2]. Cisplatin was later approved by the Food and Drug Association in 1978 after human clinical trials had been performed, and has been effective in treating testicular and ovarian cancers.

The cytotoxicity of cisplatin is due to its binding with deoxyribonucleic
acid (DNA) and the formation of DNA intrastrand cross-linkages, which interfere with cell division and lead to apoptosis. These platinum containing compounds are made up of two types of ligands referred to as the leaving and nonleaving ligands. The leaving ligands are the species in a compound that will be replaced by the DNA or protein target, whereas the nonleaving ligand will stay bound to the DNA or protein target. Cisplatin has chloride leaving ligands and amino group (NH$_3$) nonleaving ligands, which are shown in Figure 1.1.

![Figure 1.1](image)

**Figure 1.1.** The structure of cisplatin with the chloride leaving ligands and amino group nonleaving ligands bound to platinum.

Platinum compounds are thought to go across cell membranes through passive diffusion and carrier-mediated active transport [3]. One form of carrier-mediated active transport is the human copper influx transporter CTR1, which is a transmembrane homotrimer that forms a pore in cell membranes. The CTR1 pore has cysteine, methionine, and histidine amino-acid-rich domains through which the copper can then pass [4]. It is thought that cisplatin, carboplatin, and oxaliplatin can also use the CTR1
transporter to enter cells since the platinum compounds are attracted to the nucleophilic cysteine and methionine sulfur atoms, which are found in the pores formed by the CTR1 transporter. This idea is supported by experiments on mammalian cells without the CTR1 transporter that showed decreased uptake of the platinum compounds [4]. Also, the CTR1 transporter being used as a way for platinum compounds to enter cells is supported by the Hard-Soft Acid-Base Theory, which says that hard acids react more strongly with hard bases and soft acids react more strongly with soft bases. Platinum is considered a soft acid because of its high polarizability and large atomic radius, and sulfur is a soft base because of its large atomic radius and high polarizability. Since the methionine and cysteine residues in the pore formed by the CTR1 transporter have sulfur atoms, the Hard Soft Acid Base theory suggests that the platinum compounds would be likely to interact with the amino acid residues in the pore. Therefore, it is thought that CTR1 transporter is one process allowing platinum compounds to enter cells.

Cisplatin is able to travel throughout the bloodstream in its regular form shown in Figure 1.1. However, since the chloride ion concentration is low in the cytosol, water can replace the chloride leaving ligands. Therefore, the activated form of cisplatin [Pt(NH$_3$)$_2$(H$_2$O)$_2$]$^{2+}$ can act as an electrophile and bind to DNA bases forming platinum-DNA adducts [5]. Cisplatin predominantly binds to the N7 atom of the guanine base in DNA because it is a good nucleophile, which causes primarily 1,2-intrastrand crosslinking of DNA [6]. Secondarily cisplatin can also bind to the adenine nitrogenous base in DNA or to proteins, which can cause different crosslinking types. The platinum-DNA adducts formed inhibit transcription of the cancer cell’s DNA and cause apoptosis,
which is programmed cell death [8]. The potential crosslinks formed are shown in Figure 1.2 [7].

Figure 1.2. Figure showing the potential intrastrand crosslinking of cisplatin with DNA or proteins [7].

The discovery of cisplatin led to further studies into platinum-containing compounds and the Food and Drug Association approval of a cisplatin analog known as carboplatin or cis-diammine(1,1-cyclobutanedicarboxylato)platinum(II) in 1989. Carboplatin differs from cisplatin because it has a different leaving ligand, which is cyclobutane-1,1-dicarboxylic acid (CBDCA) instead of the chloride leaving ligands found in cisplatin. The structure of carboplatin is shown in Figure 1.3. The mechanism of
action of carboplatin is similar to cisplatin and involves the formation of platinum-DNA adducts leading to apoptosis.

![Figure 1.3. Structure of carboplatin with the same nonleaving ligands (NH₃) as cisplatin and a CBDCA leaving ligand.](image)

In 2002, another platinum-containing anticancer drug, oxaliplatin or (R,R)-1,2-diaminocyclohexane(oxalato)platinum(II), was also approved by the Food and Drug Association. Oxaliplatin has been most effective in treating colorectal cancer. Oxaliplatin is different from cisplatin in both its leaving ligand, which is oxalate, and its nonleaving ligand, which is diaminocyclohexane. Oxaliplatin has been shown to have a similar mechanism of action as cisplatin for killing cancer cells, and oxaliplatin forms similar platinum-DNA adducts. The structure of oxaliplatin is shown in Figure 1.4.
Figure 1.4. Structure of oxaliplatin with a diaminocyclohexane nonleaving ligand and an oxalate leaving ligand.

While all three FDA approved platinum-containing compounds have been shown to be effective in killing cancer cells, they also each have toxic side effects. These side effects include nephrotoxicity, neurotoxicity, and ototoxicity. Nephrotoxicity refers to damage to the kidneys due to toxic substances. Cisplatin has been shown to cause acute kidney injury, and the nephrotoxicity increases with a higher frequency of treatments with cisplatin [8]. Oxaliplatin has been shown to produce transient neurotoxicity in patients, which is damage to the nervous system due to toxic substances [9]. One symptom of neurotoxicity reported with oxaliplatin is peripheral neuropathy including cold dysesthesia, which is when parts of the body are extremely sensitive to cold temperatures [9]. Ototoxicity is damage to the ears due to toxic substances, and it has been shown to affect patients treated with oxaliplatin for testicular cancer by impairing
their hearing [10]. Therefore, further study into platinum-containing anticancer compounds is important to help develop more efficient and safer cancer treatments.

One important avenue of study is the toxicity effects of changing the leaving or nonleaving ligands of the platinum compounds. Since the leaving ligand becomes activated in the cytosol of the cell and is able to attack a DNA or protein target, changing the leaving ligand could potentially impact the ability of the compound to kill the cancer cells. Also, the nonleaving ligand could impact the ability of the compound to kill cancer cells since its size, charge, or stereochemistry could affect the binding of the compound to its biological target. Therefore, this research compares the toxicity of compounds with varying ligands to observe any changes in toxicity due to a particular ligand or differences in the toxicity of platinum compounds when used on different cancer cell lines.
CHAPTER 2

METHODOLOGY

Three platinum compounds were used as treatments on cancer cells. The toxicity of the platinum compounds was then determined by performing cell survival assays on the cancer cells. The three compounds tested were dichloro(ethylenediamine)platinum(II) referred to as Pt(en)Cl₂, ethylenediamine(oxalato)platinum(II) referred to as Pt(en)(ox), and oxaliplatin. Pt(en)Cl₂ has the same chloride leaving ligands as cisplatin, but a different nonleaving ligand. The nonleaving ligand in Pt(en)Cl₂ is an ethylenediamine ligand. Pt(en)(ox) has the same nonleaving ligand as Pt(en)Cl₂, and the leaving ligand in Pt(en)(ox) is the same oxalate leaving ligand that is found in oxaliplatin. Oxaliplatin was tested as well and it has an oxalate leaving ligand and a diaminocyclohexane nonleaving ligand.

Pt(en)Cl₂ was bought commercially from Sigma-Aldrich. The other two platinum compounds were synthesized as detailed below and their molecular structures were determined using a JEOL Eclipse 500 MHz NMR Spectrometer. The synthesis of silver oxalate is also described because it was used in the synthesis of both oxaliplatin and ethylenediamine(oxalato)platinum(II). The structures of Pt(en)Cl₂, Pt(en)(ox), and oxaliplatin are shown in Figure 2.1.
Figure 2.1. Structures of the platinum compounds tested including Pt(en)Cl₂, Pt(en)(ox), and oxaliplatin.

Synthesis of Platinum Compounds

Silver Oxalate:

Silver oxalate was synthesized by combining 500 mg (2.94 mmol) of silver nitrate, 150 mg (1.67 mmol) of oxalic acid, and 10 ml of deionized water in an amber vial. The mixture was stirred for 24 hours and a white precipitate formed. The solution was vacuum filtered, and ethanol was used to rinse the amber vial. The dried precipitate was collected, and the procedure yielded 319.1 mg of silver oxalate. The silver oxalate was stored in the dark.
Oxaliplatin:

First two separate solutions were made in small beakers and stirred for 5 minutes. The first solution consisted of 232 mg (0.559 mmol) of potassium tetrachloroplatinate(II) and 5 ml of deionized water, and the second solution consisted of 64 mg (0.560 mmol) of (R,R)-1,2-diaminocyclohexane and 5 ml of methanol. Next, the second solution was added dropwise to the first. The resulting solution was stirred for 24 hours and a color change occurred from red to yellow. The yellow precipitate was separated from solution using vacuum filtration. The precipitate was dried and 127.9 mg was the yield.

59.5 mg (0.157 mmol) of the yellow precipitate was combined with 47.4 mg (0.156 mmol) of silver oxalate and 35 ml of deionized water. The solution was stirred for 48 hours and then syringe filtered. A rotary evaporator was used to dehydrate the product. The synthesis of oxaliplatin yielded 37.2 mg.

Ethylenediamine(oxalato)platinum(II) or Pt(en)(ox):

Pt(en)(ox) was synthesized by combining 65.2 mg (0.200 mmol) of Pt(en)Cl$_2$ with 67 mg (0.221 mmol) of silver oxalate and 40 ml of deionized water. The solution was stirred for 24 hours in an amber vial. The white solution was then syringe filtered, and a rotary evaporator was used to dehydrate the product. The synthesis of Pt(en)(ox) yielded 80.2 mg.
Platinum Compound Toxicity Testing in Cancer Cells

Three cell lines were cultured for experiments including the colorectal cancer cell lines HT-29 and Caco-2 and the testicular cancer cell line NTERA-2. The testing was done on colorectal and testicular cancer cells since cisplatin and oxaliplatin are known to be effective at killing these cell types. The cells were purchased from the American Type Culture Collection (ATCC). The Caco-2 and HT-29 cell lines were derived from human epithelial cells of the colon from a person with colorectal adenocarcinoma [11]. The NTERA-2 cell line was derived from human epithelial cells of the testes from a person with malignant pluripotent embryonal carcinoma [11]. The Caco-2 and HT-29 cells were grown in Eagle’s Minimum Essential Medium (EMEM) with the addition of 10% fetal bovine serum, L-glutamine, and Penicillin-Streptomycin. The NTera-2 cells were grown in Dulbecco’s Modification of Eagle’s Medium (DMEM) with the addition of 10% fetal bovine serum, L-glutamine, and Penicillin-Streptomycin. The mammalian cells were cultured in an aseptic environment using standard cell culturing protocols and were stored in an incubator at 37°C and 5% carbon dioxide.

To maintain the growth of the cells, the cells were subcultured when they had grown and divided until it was visibly clear that ~75% of the plate was covered with cells. Subculturing the cells consisted of using phosphate buffered solution to rinse the cells, followed by incubating the cells with 1 ml of trypsin per 10 cm plate. Trypsin is a protease that hydrolyzes proteins at the serine residues of the easily accessible cell adhesion molecules. Therefore, adding trypsin to the cells causes the cells to loosen from
the tissue culture plate and allow the cells to be easily divided. In order to stop the function of the trypsin, 4 ml of media was added to the plate. The media has 10% fetal bovine serum, which consists of a large quantity of proteins. Therefore, adding the media will stop the trypsin from digesting the cell proteins because the fetal bovine serum will allow for an overabundance of proteins for the trypsin to digest instead of cell proteins. The cells were then counted using a Neubauer hemocytometer, which is an instrument used to count cells manually with a microscope. The hemocytometer used was a small glass instrument that held 10 \( \mu \text{l} \) of cells in media. The Neubauer hemocytometer has a defined length (1.0 mm), width (1.0 mm), and height (0.1 mm). Also, it has a grid on it that allows for the number of cells in a portion of the grid to be counted visually with the help of a microscope. Based on the number of cells counted in a portion of the grid and the known volume of the hemocytometer, the cell density can be determined. The cells were grown on 10 cm plates with a cell density of 500,000 cells/ml.

For experimental testing, the cells were counted using a hemocytometer and 500,000 cells/ml were plated onto a Greiner Bio-One 24 well cell culture plate. The cells were then incubated for 48 hours to allow the cells to adhere to the plate and start growing. The platinum compounds were diluted with media to the concentrations shown in the results section. Since each plate had 24 wells, the first three wells were control wells that had untreated cells with fresh media added so that they could continue growing in the wells. The other 21 wells were in groups where each platinum compound concentration was done in triplicate. For experimental testing, the cells were treated with 300 \( \mu \text{l} \) of the platinum compounds per well. The treatments were left on the cells for 24
hours. Then the platinum compounds were removed using a vacuum, and the MTT assays were performed.

The MTT assay included adding the MTT reagent, which is 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide salt, to the cells. The MTT reagent can be reduced by the enzymes in the mitochondria of living cells to a purple formazan. The reduction reaction and color change can only occur in living cells. Therefore, the color change can be quantified and used to determine percent cell survival.

The experimental procedure for the MTT assays included treating each well of cells with 300 µl of 0.5% MTT salt in DMEM or MEM. The MTT salt and media solution were left on the cells for 3 hours and then removed. Next, a solution was made consisting of 25 µl of Sorenson’s Buffer (0.1 M glycine, 0.1 M sodium chloride, and pH adjusted to 10.5 with sodium hydroxide) for every 200 µl of dimethyl sulfoxide (DMSO). 200 µl of the Sorenson’s Buffer and DMSO solution was added to each well. The Sorenson’s Buffer is a salt mixture that causes the cells to lyse, which releases the salts within the cells. The release of the salts inside the cells allows for the color change to be visible in the wells since the MTT salt (yellow) in dead cells or formazan (purple) in previously living cells is released.

The color change was quantified by measuring absorbance values at a wavelength of 550 nm using a Synergy H1 plate reader. Then calculations were performed to determine percent cell survival. The plate reader gave the absorbance value of each well. Since each concentration of platinum compound exposure was done in triplicate, the absorbance values for the three replicate wells were averaged. The absorbance values of
the three control wells were also averaged. The average absorbance value for each platinum compound concentration was then normalized to the three control wells’ average absorbance. The proportion of absorbance values was then converted to a percentage, which is the percent cell survival. When plating the cells the density was kept low enough to allow the cells to continue growing normally throughout the experiment. Therefore, the only cell death observed would come from treatment with a platinum compound and not from the density of cells being too high, which could inhibit cell growth.
CHAPTER 3

RESULTS

The MTT assays performed on the Caco-2, HT-29, and NTERA-2 cell lines allowed for the calculation of percent cell survival by comparing the absorbance reading of each platinum exposure to the untreated cells. Once the percent cell survival values from the MTT assays was calculated, it was graphed as a function of the concentration of the platinum compound treatment. In order to compare the toxicity of each compound used to treat the three different cell lines, the IC-50 values were determined. The IC-50 value is the inhibitory concentration where the activity of a biological process has decreased by 50%. For this research, the IC-50 values show the concentration of platinum compound needed to inhibit 50% of the activity of the mitochondrial enzymes, which convert the yellow MTT salt to a purple formazan. Therefore, a lower IC-50 value corresponds to a higher toxicity because a lower concentration of that compound can decrease the mitochondrial enzyme activity of the cancer cells by 50%. On the other hand, a higher IC-50 value corresponds to a lower toxicity. The error bars on all of the graphs correspond to the standard error.
Figure 3.1 Graph shows the results of the MTT assays on the Caco-2 colorectal cancer cells treated with Pt(en)Cl₂. The IC-50 value for Pt(en)Cl₂ is 50 μM.
Figure 3.2 Graph shows the results of the MTT assays on the HT-29 colorectal cancer cells treated with Pt(en)(ox). The IC-50 value for Pt(en)(ox) is 25 μM.

Figure 3.3. Graph shows the results of the MTT assay on the HT-29 colorectal cancer cells treated with oxaliplatin. The IC-50 value for oxaliplatin is 30 μM.
The graphs in Figures 3.1, 3.2, and 3.3 all show the results of MTT assays performed on colorectal cancer cells. Pt(en)ox had the lowest IC-50 value, which corresponds to the highest toxicity. Also, the IC-50 value was highest for Pt(en)Cl₂, which corresponds to the lowest toxicity. The IC-50 values for oxaliplatin and Pt(en)(ox) were very similar at 30 μM and 25 μM, respectively. Both Pt(en)(ox) and oxaliplatin have the same oxalate leaving ligand but differ in their nonleaving ligand.

**Figure 3.4.** This graph shows the results of the MTT assays on the NTERA-2 testicular cancer cells treated with Pt(en)Cl₂. The IC-50 value for Pt(en)Cl₂ is estimated to be between 1.5-50 μM. However, a more specific IC-50 value could not be determined from the concentrations of Pt(en)Cl₂ tested.
Figure 3.5. Graph shows the data from the MTT assay on NTERA-2 testicular cancer cells treated with Pt(en)(ox). The IC-50 value for Pt(en)(ox) is 14 μM.

Figure 3.6. Graph shows the data from the MTT assay on NTERA-2 testicular cancer cells treated with oxaliplatin. The IC-50 value for oxaliplatin is 35 μM.
The graphs shown in Figures 3.4, 3.5, and 3.6 show the results of the MTT assays performed on NTERA-2 testicular cancer cells. Pt(en)(ox) had the lowest IC-50 value (14 μM), which corresponds to a higher toxicity. Comparing between the different cell lines, Pt(en)(ox) had the lowest IC-50 value in both the testicular and colorectal cancer cells. The Pt(en)(ox) IC-50 value was 25 μM in the HT-29 cells and 14 μM in the NTERA-2 cells. Interestingly, the oxaliplatin IC-50 values were similar between the NTERA-2 cells, which had an IC-50 value of 35 μM, and the HT-29 cells, which had an IC-50 value of 30 μM.
CHAPTER 4

DISCUSSION

Following the analysis of the MTT assay data, the different leaving and nonleaving ligands have been shown to affect the toxicity of the platinum containing compounds on colorectal and testicular cancer cells. The three platinum compounds tested showed differing IC-50 values, which corresponds to different toxicity levels, between the different cancer cell lines. Therefore, the different cancer cell types had different sensitivity to the platinum compounds. A summary of the IC-50 values sorted by leaving ligand is shown in Table 4.1.

<table>
<thead>
<tr>
<th>Platinum Compound</th>
<th>Leaving Ligand</th>
<th>Cell Line</th>
<th>IC-50 Value (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt(en)(ox) Oxalate</td>
<td>HT-29</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Pt(en)(ox) Oxalate</td>
<td>NTERA-2</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Oxaliplatin Oxalate</td>
<td>HT-29</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Oxaliplatin Oxalate</td>
<td>NTERA-2</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Pt(en)Cl₂ Chloride</td>
<td>Caco-2</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Pt(en)Cl₂ Chloride</td>
<td>NTERA-2</td>
<td>~1.5-50</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.1 This is a summary chart of the IC-50 values sorted by leaving ligand.
For the HT-29 colorectal cancer cell line the Pt(en)(ox), which had an IC-50 value of 25 µM, had the lowest IC-50 value. The lower the IC-50 value the lower the concentration of the compound needed to kill 50% of the cancer cells, which corresponds to a higher toxicity. As seen in Figure 4.1, the HT-29 colorectal cancer cells Pt(en)(ox) had a similar IC-50 value to oxaliplatin, which had an IC-50 value of 30 µM. Pt(en)(ox) and oxaliplatin have the same leaving oxalate ligand, which suggests that in the HT-29 cells the oxalate leaving ligand affects the toxicity of the platinum compound.

For the testicular cell line NTERA-2 it was determined that Pt(en)(ox) also had the lowest IC-50 value, which corresponds to a higher toxicity. However, in the NTERA-2 cells the Pt(en)(ox) and oxaliplatin IC-50 values were significantly different. The Pt(en)(ox) IC-50 value in the NTERA-2 cells was 14 µM, whereas the IC-50 value for oxaliplatin in the NTERA-2 cancer cells was 35 µM. Therefore, the NTERA-2 cancer cells were 2.5 times more sensitive to Pt(en)(ox) as to oxaliplatin.

Oxaliplatin has been shown to have an IC-50 value of 1.81±1.15 µM in HT-29 cells treated with oxaliplatin for 72 hours, and an IC-50 value of 1.12±0.08 µM in NTERA-2 cells treated with oxaliplatin for 72 hours [12]. The experimental IC-50 values determined from the MTT assays are higher because the cells were treated for a shorter period of time. The ability of the platinum compounds to kill cancer cells is thought to depend on the amount of the platinum compound that accumulates in the cell, which causes intrastrand crosslinking with DNA. Therefore, for the 24 hour treatments the IC-50 values could have been higher because less of the platinum compounds were able to accumulate in the cancer cells and cause apoptosis during the narrow time window.
Also, the Caco-2 colorectal cancer cells treated with Pt(en)Cl$_2$ had an IC-50 value of 50 $\mu$M, which is higher than both the Pt(en)(ox) and oxaliplatin IC-50 values in the HT-29 colorectal cancer cell line. Therefore, the Caco-2 cancer cells had a higher sensitivity to the Pt(en)Cl$_2$ than the HT-29 cancer cells had to Pt(en)(ox) or oxaliplatin. For the NTERA-2 cells treated with Pt(en)Cl$_2$ the specific IC-50 value was not determined based on the data collected. At a 50 $\mu$M concentration of Pt(en)Cl$_2$ the percent cell survival was 30%. However, the NTERA-2 cancer cells treated with oxaliplatin never dropped below 40% cell survival even at the 60 $\mu$M concentration. Therefore, the NTERA-2 cancer cells seem to be more sensitive to Pt(en)Cl$_2$. Pt(en)Cl$_2$ has the same chloride leaving ligands as cisplatin, and in the literature IC-50 values on NTERA-2 cells treated for 72 hours the same trend is seen between cisplatin and oxaliplatin IC-50 values. For the literature IC-50 values of NTERA-2 cancer cells treated for 72 hours, cisplatin had an IC-50 of 0.14±0.03$\mu$M and oxaliplatin had an IC-50 value of 1.12±0.08 $\mu$M [12]. Therefore, the literature IC-50 values show NTERA-2 cancer cells being more sensitive to cisplatin than to oxaliplatin, and the experimental results show the NTERA-2 cancer cells showing the same trend of being more sensitive to the compound with the same chloride leaving ligands as cisplatin.

Overall, it was shown that the toxicity of the platinum compounds differed between cell lines and that the compound with the highest toxicity in both cancer cell types was Pt(en)(ox). Pt(en)(ox) had a lower IC-50 value and higher toxicity in both cell lines than the therapeutic standard drug treatment, which is oxaliplatin. The NTERA-2 cells were 2.5 times more sensitive to Pt(en)(ox) than to oxaliplatin. The literature IC-50
values were lower after the cancer cells had been treated for 72 hours than the experimentally determined IC-50 values, which were determined after treating the cancer cells for 24 hours. This supports the belief that cancer cell death is dependent upon the amount of the platinum compound that accumulate in the cancer cells, which leads to intrastrand crosslinking with DNA and apoptosis. In conclusion, the platinum compounds had differing toxicities based on their leaving and nonleaving ligands, and the cancer cell lines showed different sensitivities to the same compounds.
Works Cited


