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Research Article

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EFFECTS OF CARBOPLATIN AND CISPLATIN ON C33A CELL LINE

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ABSTRACT

Platinum-based chemotherapeutic drugs such as Carboplatin and Cisplatin are widely used in cancer treatments due to their cytotoxic effects on Cancer cells. This Paper mainly focuses on the study of the effect of drugs on a C33A cell line in the commonly used Cervical cancer model. This study employs two independent methodologies namely MTT Assay for determining cell viability and Molecular Docking targeting Human Peroxiredoxin 2 to predict the predominant binding affinity. This study aims to provide insights into the drug's efficacy and potential differences in their impact on the cancer cell line. Moreover, the Molecular Docking approach is employed to investigate the interaction between carboplatin and cisplatin with human peroxiredoxin 2 (Prx2) a protein involved in redox regulation and implicated in cancer progression. The study's Molecular docking aspects focus on the interaction between the two drugs with Human Prx2. Peroxiredoxin 2 is chosen as the docking target due to its significance in redox homeostasis and may play a role in the way cells react to drugs based on platinum, it was selected as the Docking target. A thorough understanding of the cellular response to carboplatin and cisplatin is made possible by the integration of the MTT assay result withMolecular docking data which also brings information on possible mechanisms of action. This study provides our knowledge and understanding of platinum-based chemotherapyand potentially impacts future advancements in the treatment of cervical cancer and related diseases.

Keywords: MTT Assay, Molecular Docking, Human Peroxiredoxin 2, Platinum-basedDrug.

INTRODUCTION

Globally, among women, cervical cancer is the fourth most common cancer. In cervical cancer, many cell lines are isolated from human cervical carcinoma among which C33A is one of the HPV-negative cervical cancer cell lines. Human papillomavirus (HPV) is an infection caused by a DNA virus. The C33A cell linehas been instrumental in advancing our understanding of cervical cancer and providing a platform for investigating various aspects of Cancer biology. There is still an endless search for more specific and effective therapeutic interventions, despite developments in medical research and treatment modalities. Chemotherapeutic agents based on platinum, specifically carboplatin and cisplatin, have become essential for treating a range of cancers, including cervicalcancer. They have become essential elements of clinical oncology due to their effectiveness in causing apoptosis and preventing the growth of cancer cells. Even though carboplatin and cisplatin share a common mechanism of action, significant differences in their chemical structures and pharmacokinetics may result in distinct cellular responses. It is critical to understand all aspects of their effects on particular cancer cell lines to maximize therapeutic outcomes and minimize side effects (Keepers YP, *et al.,* 1991). This paper explores the complex interactions between the well-established C33A cell line, used in cervical cancer research, and carboplatinand cisplatin. The study of the mechanisms of cellular growth is fundamental to understanding the intricate processes governing cell proliferation, viability, and response to external stimuli. Among the various methodologies available, the MTT (3-(4,5-dimethylthiazol2-yl)-2,5-diphenyltetrazolium bromide) assay stands out as a versatile and widely employed tool in cellular research. The MTTassay, introduced by Mosmann in 1983, is based on the reduction of the yellow tetrazolium salt MTT to formazan crystals by mitochondrial dehydrogenases in viable cells. This reduction is indicative of cellular metabolic activity and serves as a reliable measure of cell viability. The assay's simplicity, sensitivity, and reproducibility make it a cornerstone in cell biology research, providing quantitative data that can be applied across diverse experimental conditions (Vajrabhaya L *et al.,* 2016). This paper develops into the comprehensive analysis and identification of the growth analysis of the C33A cell line, derived from cervical carcinoma, utilizing the MTT assay. The C33A cell line, known for its relevance in cervical cancer research, serves as a valuable model to investigate cellular responses under different experimental conditions. The investigation is approached through a combination of the MTT assay, evaluating cellular viability, and molecular docking simulations targeting human peroxiredoxin 2 (Prx2). The MTT assay serves as a fundamental tool for evaluating cell viability and cytotoxicity in response to drug exposure. By estimating the concentration-dependent effects of carboplatin and cisplatin on C33A cell viability, this study aims to provide quantitative insights into the drug's efficacy (Wilson, J.K *et al.,* 1990). The MTT assay results will be crucial in establishing dose-response relationships, aiding in the identification of optimal drug concentrations forpotential clinical applications. Meanwhile, molecular docking simulations targeting human Prx2 will be used to investigate the intricate molecular interactions between carboplatin and cisplatin. Peroxiredoxin 2, a protein involved in redox homeostasis, has been linked to cancer progression, making it an important target for understanding how platinum-based drugs work (Mosmann, T., 1983). Within thePrx2 binding site, molecular docking will reveal potential binding affinities, binding modes, and conformational changes induced by carboplatin and cisplatin.Furthermore, the study's focus on human Prx2 in molecular docking simulations contributes an additional level of importance. Potential pathways through which

these platinum agents exert their anticancer effects may be revealed by analysing how they interact with a protein involved in redox regulation, such as Prx2. This understanding of the molecular level may direct future efforts to develop drugs, ultimately leading to the improvement of targeted therapies (Gory, A.H et al., 1991).

MATERIALS AND METHODS

DMEM Medium (Dulbecco's Modified Eagles Medium), Trypsin, PBS Buffer (Phosphate buffered saline), FBS (Fetal Bovine Serum), Sodium bicarbonate, MTT dye, Milliq Water, DMSO (Dimethyl Sulfoxide) and Antibiotics and software like Auto Dock 4.2 and GraphPad Prism (student trail).

CELL GROWTH AND PROLIFERATION

The C33A cell line was grown in the T25 flask using the DMEM medium with 10%FBS and antibiotics. After reaching 80% confluency cells were subcultured into 3 new T25 flasks and were incubated in a 5% co_2 incubator until reached their confluency (Berridge, M.V *et al.,* 1993).

PROCEDURE

90-100% confluency T-25 flask was taken and aspirated the media(DMEM). The trypsin was added (depending on the confluency) into it. Then incubated in a 5% Co2 incubator at 37˚c for 6-7 mins. The media was resuspended for 10 times. Media should be put into a new falcon tube. Then centrifuge the tube for 6 mins the pellet was taken and the supernatant was aspirated. By the creation of a pellet, the media was added to the falcon tube, and the media was resuspended 5 times without the formation of clumps. Then, using a micropipette, 10ul of the medium containing cells were added to the hemocytometer, and the cells were examined in the microscope. Then cells were calculated.

Total cells/ml = (Total cells counted x Dilution factor x 10,000 cells/ml)/Number of squares.

Average no of cells =105.7

Total cells/ml $=105.7\times10^4\times1$

$= 1050000.$

Now in two 96-well plates, 100µl media was added with a cell volume of 5000 cells per well (only 60 wells) and incubated for 24 hours in an incubator.

DRUG TREATMENT

The drug (Carboplatin and Cisplatin) was diluted in a media as per the calculation (Table 1). 96well plates were taken from the incubator and the media was discarded. Then the media containing the drug (100µl) was transferred from the Eppendorf to each well. And placed in an incubator for 48 hours. After 48 hours of incubation, the 96 well plate was taken and aspirated the media with a multi-chamber pipette. MTT was prepared by taking 7.5 gm of MTT and dissolved in 14 ml of DMEM (Sun Y *et al*., 2009). 100µl of dye was added to each well of the plate. Incubated it for 4 hours in a 5% Co2 incubator. The plates are taken out of the incubator, the dye is aspirated, and formazan crystals are seen under a microscope. The wells were then

S.no	Desired concentration (µm)Carboplatin	Desired concentration (µm)Cisplatin	Drug(µl) of Cisplatin	Drug(µl) of Carboplatin	Media (ml) of Cisplatin	Media(ml) of Carboplatin

added with 50µl of DMSO, and Checked for complete solubilization of the purple formazan crystals and measured the spectrophotometric absorbance of the samples using a microplate (ELISA) reader. The filters that are available for the ELISA reader that are being used indicate that the wavelength to measure the formazan product's absorbance is between 450 and 630 nm**.**

MOLECULAR DOCKING

STEP **I**: Protein Structure Refinement:

Human peroxiredoxin 2 protein was downloaded from the RCSB Protein Data Bank(PDB). PDBID – 7KJ1 and the protein was refined in this step.

STEP **II:** Ligand Preparation:

The desired Ligand was downloaded from a PubChem in an SDF file format and then it was converted in Open Babel to PDB file format.

The protein and ligand files that are prepared by the above said procedures are taken for docking.

STEP **III:** Docking with Auto Dock 4.2:

The Protein and the Ligand are prepared in Auto Dock and the grid file is created by saving it as the 'gpg' file. By running the Grid file the 'glg' file is automatically created and saved. The dock file was also created and saved as a 'dpf' file (Monsuur HN *et al.,* 2016). The running of the Dock file will create a 'dlg' file and automatically save after running successfully.

TABLE 1: Drug Calculation

1	50	25	5.3	1.8	694.7	998.2
2	100	50	10.6	3.7	689.4	996.3
3	150	75	15.9	5.5	684.1	994.5
4	200	100	21.2	7.4	678.8	992.6
5	250	125	26.5	9.2	673.4	990.8
6	300	150	31.8	11.2	668.4	988.8
7	350	175	37.1	13	662.9	987
8	400	200	42.4	14.8	657.6	985.2
9	500	225	47.7	18.5	652.3	981.5

STEP **III:** Analysing the Docking result:

The docking result was analyzed in Auto Dock 4.2 to visualize the docked conformation and analyse the interaction.

RESULTS AND DISCUSSIONS



Fig 1: 96 well plate after the purple formazan crystals are formed.

The observed calculation on 96 well plates was used to calculate the IC50 value by using Graph Pad Prism (student trial version).

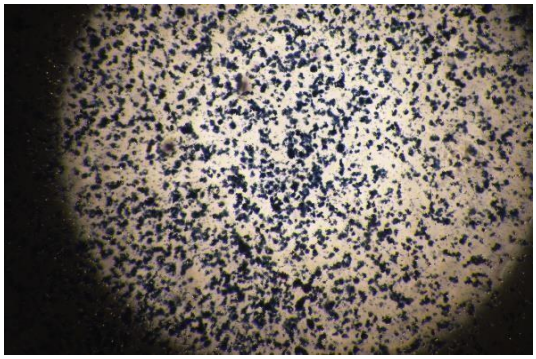


Fig 2: Cells in a well after added MTT dye the formation of formazan crystal.

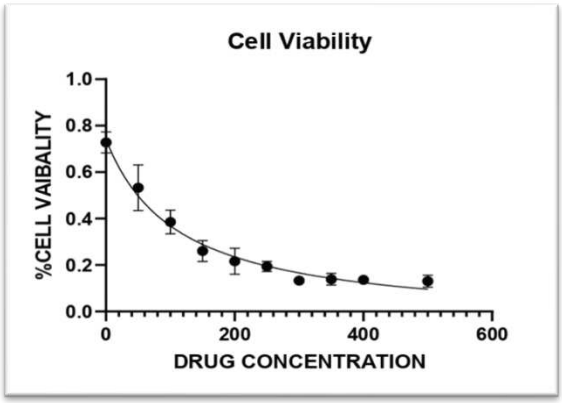


Fig 3: Graph for Cell viability by Graph Pad Prism of Carboplatin

TABLE 2: Absorbance of Carboplatin in 96 well plate by ELISA plate reader

C (X)	0	50	100	150	200	250	300	350	400	500
R1 (Y)	0.773	0.556	0.447	0.347	0.298	0.236	0.163	0.176	0.168	0.152
R(Y)	0.695	0.502	0.472	0.27	0.324	0.248	0.19	0.226	0.187	0.17
R3 (Y)	0.825	0.54	0.374	0.244	0.186	0.221	0.146	0.148	0.164	0.144
R4 (Y)	0.756	0.676	0.424	0.357	0.228	0.254	0.192	0.184	0.198	0.186
R5 (Y)	0.786	0.719	0.368	0.322	0.306	0.267	0.2	0.193	0.193	0.22
R6 (Y)	0.801	0.47	0.496	0.287	0.221	0.206	0.174	0.175	0.175	0.177

TABLE 3: Inhibition and IC50 values ofCarboplatin for cell viability of 48hrs using Graph Pad Prism.

Note C- concentration R- response

Inhibitor vs. response (three parameters)	Values
Best-fit values:	
Bottom	-0.04915
Top	0.7410
LogIC50	2.050
IC50	**112.2**
Span	0.7901
95% CI (profile likelihood):	
Bottom	-0.1126 TO 0.003685
Top	0.7022 TO 0.7800
LogIC50	1.939 TO 2.166
IC50	86.89 TO 146.5
Goodness of Fit:	
Degrees of Freedom	57
R squared	0.9311
Sum of Squares	0.1418
Sy.X	0.04987
Number of points:	
# of X values	60
# Y values analysed	60

TABLE 4: Absorbance of Cisplatin in 96 well plate by ELISA plate reader

C(X)	0	50	100	150	200	250	300	350	400	500
R1 (Y)	0.773	0.556	0.447	0.347	0.298	0.236	0.163	0.176	0.168	0.152
R2 (Y)	0.695	0.502	0.472	0.27	0.324	0.248	0.19	0.226	0.187	0.17
R3 (Y)	0.825	0.54	0.374	0.244	0.186	0.221	0.146	0.148	0.164	0.144
R4 (Y)	0.756	0.676	0.424	0.357	0.228	0.254	0.192	0.184	0.198	0.186
R5 (Y)	0.786	0.719	0.368	0.322	0.306	0.267	0.2	0.193	0.193	0.22
R6 (Y)	0.801	0.47	0.496	0.287	0.221	0.206	0.174	0.171	0.175	0.177

TABLE 5: Inhibition and IC50 values of Cisplatin for cell viability of 48hrs using Graph Pad Prism

Inhibitor vs. response (three parameters)	Values
Best-fit values:	
Bottom	0.1065
Top	0.8037
LogIC50	0.9835
IC50	**9.626**
Span	0.6972
95% CI (profile likelihood):	
Bottom	0.07603 to 0.1348
Top	0.7587 to 0.8487
LogIC50	0.7909 to 1.141
IC50	6.179 to 13.83
Goodness of Fit:	
Degrees of Freedom	57
R squared	0.9288
Sum of Squares	0.1729
Sy.X	0.05507
Number of points:	
# of X values	60
# Y values analysed	60

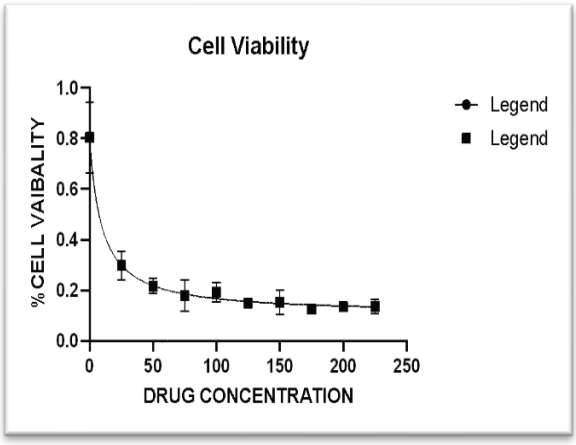


Fig 4: Graph for Cell viability by Graph Pad Prism of Cisplatin.

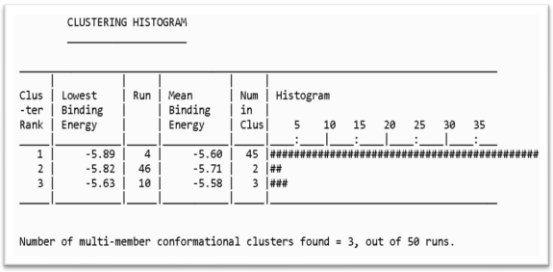
DOCKING RESULTS

Molecular docking is a computational method used in structural biology and drug discovery to predict the preferred orientation and binding affinity of a small molecule (ligand) to a target protein. This technique is appreciatiable in understanding the molecular basis of protein-ligand interactions, predicting binding modes, and identifying potential drug candidates. Here's an overviewof the molecular docking process.

BINDING AFFINITY IN CARBOPLATIN

The docking study is to predict a particular biological activity based on the binding orientation/affinity of the small molecule (carboplatin) to the target molecule (Human Peroxiredoxin 2). In the docking study, the binding energy was predicted in terms of the interaction energy (kcal/mole) (Paul CD *et al*., 2016). The docking went through 50 different runs among which the strongest bond between the Prx2 and Carboplatin is based on the highest binding energy (He P *et al*., 2023).

The highest binding energy of the complex is **-5.89** on the **4th** run.



CLUSTERING HISTOGRAM

Clus-ter Rank	Lowest Binding Energy	Run	Mean Binding Energy	Num in Clus	Histogram 5 10 15 20 25 30 35
1	-5.89	4	-5.60	45	##
2	-5.82	46	-5.71	2	##
3	-5.63	10	-5.58	3	###

Number of multi-member conformational clusters found = 3, out of 50 runs.

Fig 5: Clustering Histogram of Carboplatin

BINDING AFFINITY IN CISPLATIN

The docking study is to forecast a particular biological activity based on the binding orientation/affinity of the small molecule (cisplatin) to the target molecule (Human Peroxiredoxin 2). In the docking study, the binding energy was predicted in terms of the interaction energy (kcal/mole) (Doroshow, J. H. *et al*., 1983). The docking went through 50 different runs among which the strongest bond between the Prx2 and Carboplatin is based on the highest binding energy.

The highest binding energy of the complex is **-3.60** on the **37th** run.

CLUSTERING HISTOGRAM

Clus-ter Rank	Lowest Binding Energy	Run	Mean Binding Energy	Num in Clus	Histogram 5 10 15 20 25 30 35
1	-3.60	37	-3.40	23	#######################
2	-3.35	27	-3.17	12	############
3	-2.90	32	-2.90	1	#
4	-2.63	33	-2.53	11	###########
5	-2.36	8	-2.36	1	#
6	-2.32	23	-2.28	2	##

Number of multi-member conformational clusters found = 4, out of 50 runs.

Fig 6: Clustering Histogram of Cisplatin

VISUALIZATION OF CISPLATIN AND CARBOPLATIN

Displaying 2D interaction diagrams (Fig 7a&b) helps to understand the specific interactions between the ligand and the target at the atomic level. This can include hydrogen bonding, van der Waals interactions, and other types of bonds (Tacar, O *et al*., 2013). When visualizing molecular docking results, it's crucial to consider the biological context and validate the results through experimental method (Kamalambigeswari R *et al*., 2019). Visualizing 3D molecular interactions (Fig 8a&b) is crucial for understanding the binding modes and key interactions between ligands and target proteins.

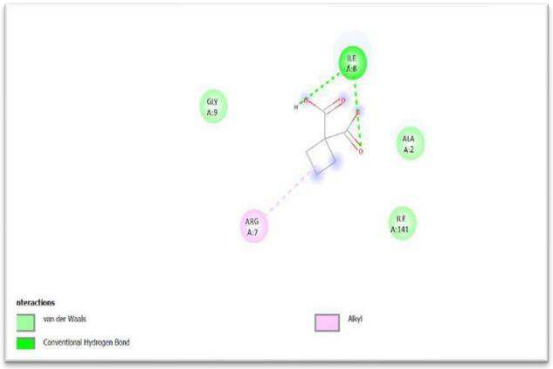


Fig 7a: 2D Visualization of Carboplatin

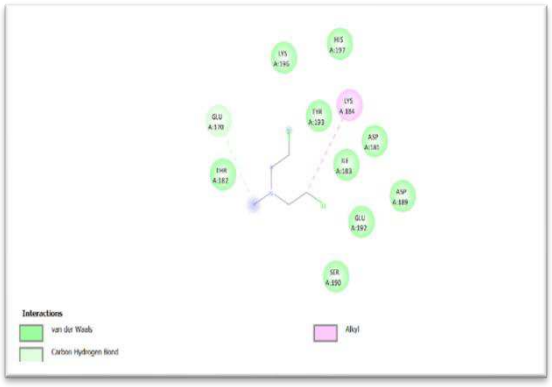


Fig 7b: 2D Visualization of Cisplatin

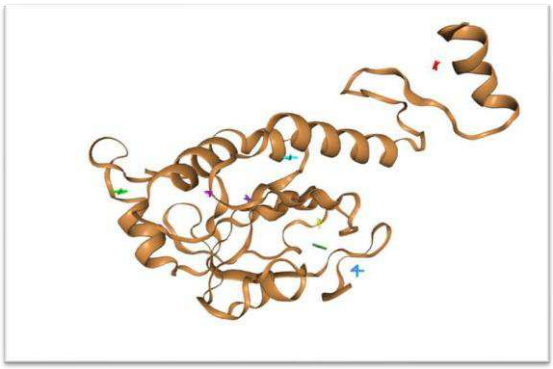


Fig 8a: 3D Visualization of Cisplatin

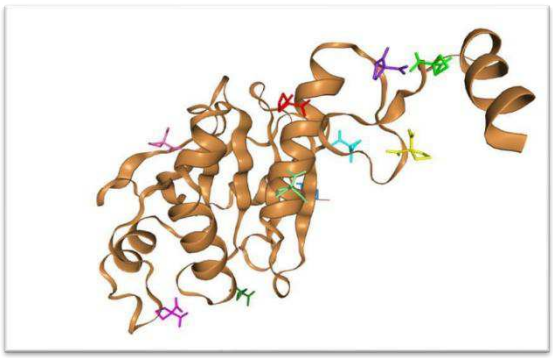


Fig 8b: 3D Visualization of Carboplatin

INTERMOLECULAR INTERACTION

Hydrophobic Interactions

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	173A	PRO	3.90	1542	1338

Hydrogen Bonds

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	177A	LYS	3.24	4.00	135.00	✓	×	1363 [Nam]	1546 [O.co2]
2	180A	SER	2.16	2.77	119.51	✓	✓	1388 [O3]	1546 [O.co2]
3	181A	ASP	2.22	3.08	146.05	✓	×	1389 [Nam]	1549 [O.co2]

Salt Bridges

Index	Residue	AA	Distance	Protein positive?	Ligand Group	Ligand Atoms
1	197A	HIS	4.04	✓	Carboxylate	1550, 1549

Fig 9a: Interaction Between the Residues to different types of Bonds incarboplatin

Halogen Bonds

Index	Residue	AA	Distance	Donor Angle	Acceptor Angle	Donor Atom	Acceptor Atom
1	190A	SER	3.66	142.62	90.32	1968 [Cl]	1861 [O2]

Salt Bridges

Index	Residue	AA	Distance	Protein positive?	Ligand Group	Ligand Atoms
1	170A	GLU	4.36	×	Tertamine	1961
2	181A	ASP	5.35	×	Tertamine	1961

Fig 9b: Interaction Between the Residues to Different Types of Bonds in Cisplatin.

CONCLUSION

MTT Assay events the ability of cells to convert the MTT reagent into a purple formazan product, with the amount of formazan formed directly proportional to the number of viable cells. This Assay evaluated the cell viability at lower concentrations with carboplatindrug on the C33A cell line. MTT Assay results involve measuring the absorbance of the formazan product, which can be quantified using a spectrophotometer. The results are often presented in terms of the optical density (O.D) or absorbance at a wavelength, commonly 430nm/650nm (Prasada Rao CM et al., 2023). The calculated **IC50** value of carboplatin is **112.2** and the **IC50** value of cisplatin is **9.6** which further characterizes the C33A cell line's response to the experimental condition. Therefore, based on the viability assay Cisplatin is more efficient even at low concentrations than Carboplatin. The half-maximal inhibitory concentration of Cisplatin is lower than that of Carboplatin. The quantitative dimension to the cytotoxic effects of Carboplatin and Cisplatin on the C33A cell line makes this study more informative for researchers and clinicians in the field of genetic engineering and cancer therapy (Akash S *et al*., 2023).

In this study of Molecular Docking the binding affinity is calculated to find the strongest binding between the protein and ligand molecule. In study the combination of docking program and scoring function can be useful for the better estimation of the binding affinity. Therefore, in this study compare to Cisplatin the Carboplatin has highest binding energy so it signifies a strong and stable interaction between the ligand and protein molecule Human peroxiredoxin 2.

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Cell line source: ATCC

Consent to Participate: Not applicable

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