

## The Role of Cisplatin Loaded Biocompatible Nanoparticles in Cancer Treatment

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### Research Article

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### ABSTRACT

Chemotherapeutic treatments focusing on cisplatin, ovarian cancer, testicular cancer, breast cancer, colon cancer, lung cancer etc. seen in adults and children. Cisplatin is an antineoplastic agent frequently used in cancer types. Drug resistance to cisplatin has an effect on the success of the treatment. After cisplatin-administered treatments, patients may experience cisplatin-induced side effects of various toxic dimensions, such as nausea, nephrotoxicity, cardiotoxicity, hepatotoxicity, and neurotoxicity. As a result of the literature review, various studies have been carried out to reduce the side effects and toxic effects of cisplatin and other anticancer molecules without interfering with their activities. In this study, we planned to prepare a cisplatin-loaded chitosan nanoparticle formulation based on the ionic gelation method using chitosan and tripolyphosphate (TPP) with the information obtained from the literature. In this study, chitosan nanoparticles containing the same concentration of cisplatin were applied to MDA-MB-231 breast cancer cell lines obtained from the American Type Culture Collection (ATCC). As a result, biocompatible nanoparticles with similar cytotoxic effects cause less side effects in other cells and tissues than other nanoparticles.

**Keywords:** Cancer, Cisplatin, Chitosan, Nanoparticle, Cytotoxicity.

## Introduction

Cancer is one of the most important diseases that visibly affects living standards, requires long-term heavy treatment stages, has a very high mortality and morbidity rate, requires special care after diagnosis, and threatens human health today [1]. Many types of cancer usually begin with the uncontrolled division and proliferation of abnormal cells. Cancer types are mostly named according to the tissues and organs from which they originate [2]. Cancer cells accumulate over time to form tumors. Tumors may be benign or malignant according to their activity. Tumors that cause cancer are malignant tumors. The cells of such tumors are abnormal. Depending on their size, these tumors have the ability to compress, penetrate and destroy normal cells. When cancer cells leave the tumor of origin, they can reach the rest of the body through the blood or lymph circulation. They form colonies in these regions and continue their growth processes. The spread of cancer cells to the body in such a way is called metastasis, that is, the spread of cancer to other tissues and organs [3]. Patients with cancer are treated with either surgical treatment methods such as surgical interventions or newer treatment methods such as gene therapy, depending on the type of cancer and its stage. Radiation therapy is used dangerous doses of radiation to shrink or kill cancerous cells [4-6]. In addition to these, many drugs and drug active substances such as cisplatin are used in the treatment of cancer. Cisplatin is one of the first metal-based, widely used chemotherapeutic agents [7].

Cisplatin is one of the most widely used chemotherapeutic drugs for various tumors in the clinic due to its high activity and broad spectrum of action. [8, 9]. Cisplatin is an important chemotherapeutic agent widely used in the treatment of various diseases such as testis, ovary, breast, cervical, prostate, bladder, and lung [10]. Cisplatin is formed by the coordination of a central platinum atom in the "cis" position, two chlorine and two ammonia molecules. Molecular structure of cisplatin was shown in Figure 1.

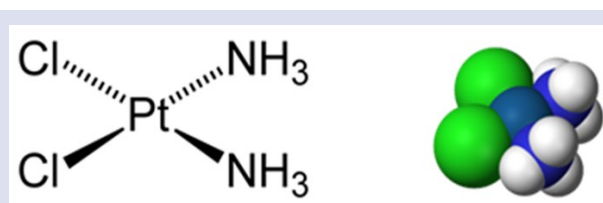


Figure 1. 2-dimensional and 3-dimensional structure of cisplatin [11]

Cisplatin generally binds to DNA from its genomic region to form gDNA or binds from its mitochondrial region to form mtDNA. It causes lesions in DNA. By acting on DNA, mRNA and proteins, it causes their inhibition and prevents their production. It activates multiple transduction pathways by interfering with the DNA replication mechanism. These events eventually lead to apoptosis and necrosis. [12-13]. Nephrotoxicity,

ototoxicity, gastrointestinal, neurotoxicity and hepatotoxicity often occur after cisplatin treatment [14]. It is known that cisplatin causes nephrotoxicity by contributing to the formation of free oxygen radicals such as superoxide and hydroxyl radical. Platinum response to combined cisplatin use is primarily high, however, many cancer patients will eventually resurface with cisplatin-resistant diseases. Therefore, significant resistance to the drug has been observed in many patients whose condition relapses due to cisplatin therapy. Suggested cisplatin-resistant mechanisms include differences in uptake and flux of cisplatin into cells, increased biotransformation and detoxification in the liver, and increased over DNA repair and anti-apoptotic mechanisms [15]. Cisplatin treatment is very beneficial on the life expectancy of breast cancer patients [16].

The MDA-MB-231 cell is an epithelial line of human breast cancer cells. Metastatic breast and adenocarcinoma from pleural effusion of a woman and is one of the breast cancer cell lines frequently used in research laboratories [17]. Many risk factors that cause breast cancer have been identified. Among these risk factors, hereditary factors are very important in the formation of breast cancer. Approximately 40% of human breast cancers are caused by mutations in the p53 gene [18]. In addition, cancer patients experience physical collapse such as nausea, loss of appetite, weight loss, hair loss, and psychological collapse such as depression and depression as a kind of side effect during chemotherapy treatment. These treatments are provided with psychological support [19]

Nanoparticles are used in cancer treatment. In general, as a result of the studies chitosan inhibits cell proliferation, induces apoptosis and has been shown to reduce its size. Therefore, it is widely used in breast cancer. Chitosan has many advantages such as bioavailability, biocompatibility, biodegradability, and mucoadhesiveness. It is distinguished from other nanoparticles by its properties [20]. It also has features such as antibacterial, antifungal, antitumor and hemostatic activities. It stimulates wound healing and immune system. Chitosan is one of the natural polysaccharides obtained from the shells of shellfish and fungal cell walls and is obtained as a result of deacetylation of chitin [20, 21]. Molecular structure of chitosan was shown in Figure 2 [22].

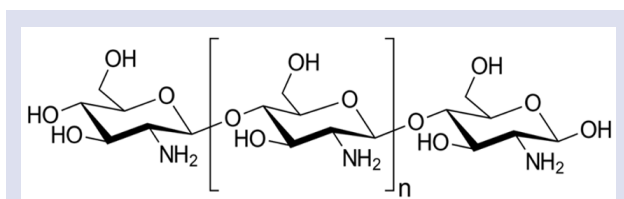


Figure 2. The structure of chitosan [22]

In this study, it was aimed to prepare nanoparticles with chitosan, a biocompatible polymer and cisplatin,

which has a cytotoxic effect on cancer cells. As seen in the studies, it has been stated that cisplatin has various toxic and side effects together with its use in cancer treatment. Our hypothesis is that the preparation of chitosan nanoparticles containing cisplatin in the concentration it is applied alone and that the biocompatible nanoparticles on cells and have more effective cytotoxic effect on MDA-MB-231 cancer cells.

## Materials and Methods

### Chemicals and Medical Consumables

MDA-MB-231 breast cancer (HTB-26™) cell line, penicillin/streptomycin (10,000U/mL), DMEM/Nutritional Mixture, Fetal Bovine Serum (FBS), Trypsin-EDTA solution and various consumables required for cell culture were used. In the preparation of chitosan nanoparticles, medium molecular weight chitosan, tripolyphosphate (TPP), glacial acetic acid were used.

### Preparation of Chitosan Nanoparticles

The ionic gelation method was used for the preparation of nanoparticles. It were dissolved in 0.05 % acetic acid, with a medium molecular weight chitosan concentration of 2,5 mg/ml. The dissolution process of chitosan was performed using a magnetic stirrer. The dilution of acetic acid was done with sterile bidistilled water. TPP was dissolved in sterile bidistilled water with stirring on a magnetic stirrer at a concentration of 2,5 mg/ml. The determined concentration of cisplatin were added to the dissolved TPP and mixed for 10-15 minutes to distribute it homogeneously. Afterwards, TPP containing cisplatin were dropped into the chitosan mixed in a magnetic stirrer with a certain dropping rate, and the mixing process were continued for 3 hours after the dropping process. Then, the chitosan-TPP mixture were added to a 50 ml centrifuge tube and centrifuged at 12.000 rpm for 15 minutes. After centrifugation, discarding the supernatant in the upper part, sterile bidistilled water were added and centrifugation were taken place again. After this process was repeated three times, the chitosan nanoparticles containing cisplatin at the bottom of the tube were maintained at -20 °C overnight. The next day, our frozen nanoparticles were lyophilized in the lyophilizer. Our lyophilized nanoparticles were put into an eppendorf tube and stored in a moisture-free environment after passing through the sieves used in nanoparticle preparation. Mechanical characterization studies of nanoparticles were performed using Malvern Zetasizer device [23].

### Cell Culture

MDA-MB 231 cells obtained from American Type Culture Collection (ATCC) were in an incubator at 37°C and 5% CO<sub>2</sub>, in flasks, in DMEM cell culture medium including 1% L-glutamine and penicillin-streptomycin, 10% fetal bovine serum were reproduced in vitro condition. When the cells reach a certain density (80%), the cells were passaged and work were started after a certain passage.

### XTT Cell Viability Assay

The effect of cisplatin and cisplatin-loaded nanoparticles on the viability of MDA-MB 231 cell line were investigated with the XTT cell viability assay. In this method, metabolically active cells reduce XTT, a tetrazolium salt, to orange colored formazan crystals. Because of the dye was water-soluble, the dye density can be read at certain wavelengths (450 nm) with the use of a spectrophotometer. The dye density in orange is proportional to the number of metabolically active cells. For cytotoxicity experiments, first of all,  $10 \times 10^3$  cells were taken from the medium containing 100  $\mu$ L of DMEM + 10% FBS + 1% antibiotic, and cells were seeded in a sterile 96-well microplate and incubated overnight for cells to adhere. The next day, the medium on the cells were removed, the wells were washed with PBS, fresh medium were added to the cells, different concentrations of cisplatin were applied to the cells and incubated for 24 hours. At the end of this period, the medium were removed and the cells were washed three times with PBS. Then, 100  $\mu$ l of clear (colorless) DMEM and 50  $\mu$ l of XTT solution were added to each well and incubated in a CO<sub>2</sub> incubator for 4 h. After the incubation, the optical density value were read at 450 nm in the microplate reader, the cell viability rate of the control group were considered as 100% [24].

### Results and Discussion

#### Mechanical Characterization Result of Nanoparticles

The zeta potential is known as the electrostatic interactions between particles and cells in a fluid medium. According to the results, the zeta potential values of the nanoparticles ranged between  $2.36 \pm 0.03$  mV and  $2.58 \pm 0.02$  mV (Table 1). Thanks to the positive zeta potential of nanoparticles, it is easier to adhere to the negatively charged cell membrane and enter the cell through receptors. The zeta potential values of the nanoparticles obtained at the end of the study can be evaluated as suitable for application. Particles above a certain molecular size are not able to pass through the cell membrane and show the desired effects in the target region. For this reason, it is desired that the nanoparticles have size in the specified ranges ( $< 400$  nm). In this study, nanoparticle sizes were observed to be between  $312.14 \pm 1.8$  nm and  $336.25 \pm 2.2$  nm. It can be concluded that nanoparticle sizes are appropriate for in vitro cell culture assay, hence its antiproliferative effect may be within the expected values in in vitro studies. The polydispersity index value is a significant parameter in the homogeneity of the size distribution of the nanoparticle and in the evaluation of the aggregation risk. This value is required to be less than 0.4 in terms of application.

Table 1. Particle size, zeta potential and polydisperse index values of chitosan nanoparticles

Formulations (Chitosan- TPP nanoparticles)	Zeta potential(mV) $\pm$ SD	Size (nm) $\pm$ SD	Polydispersity index $\pm$ SD
*NP1	$2.46 \pm 0.04$	$312.14 \pm 1.8$	$0.242 \pm 0.02$
*NP2	$2.58 \pm 0.02$	$334.22 \pm 2.6$	$0.273 \pm 0.04$
*NP3	$2.36 \pm 0.03$	$336.25 \pm 2.2$	$0.258 \pm 0.03$

\*All nanoparticles (NP1, NP2, NP3) containing medium molecular weight chitosan, tripolyphosphate and cisplatin.

When we evaluate the results, it can be said that the polydisperse index values of nanoparticles are lower than 0.4 and it is suitable for application. According to the results of the mechanical characterization study, the NP1 formulation has the most suitable particle size, zeta potential and polydisperse index values, so its applicability is higher in terms of in vitro studies.

#### Antiproliferative Activity Result of Cisplatin and Nanoparticles Loaded With Cisplatin

Nanoparticles loaded with cisplatin and only cisplatin were treated to the MDA-MB 231 cells at concentrations (2.5  $\mu$ g/ml, 5  $\mu$ g/ml, 10  $\mu$ g/ml, 25  $\mu$ g/ml, 50  $\mu$ g/ml) and the cytotoxic efficiencies and IC50 values of the samples were calculated and evaluated. Nanoparticles used in in vitro studies were prepared using the same method but mechanical features such as particle size, zeta potential and polydispersity index may differ owing to the differences arising from the experimental environment and minor mistake. Due to this difference, the antiproliferative activities of nanoparticles may also show slight differences. Concentration-dependent MDA-MB 231 antiproliferative activity of samples including only

cisplatin was calculated as 74.36 % at 2.5  $\mu$ g/mL concentration and 47.28 % at 50  $\mu$ g/mL concentration. Cell viability of NP1 samples 64.28 % at 2.5  $\mu$ g/mL concentration and 41.23 % at 50  $\mu$ g/mL concentration. If we evaluate the cell viability of NP2 samples on MDA-MB 231 cells, cell viability ranged between 68.87 % and 43.36 % depending on the concentration. In addition, cell proliferation of NP3 samples 69.22 % at least concentration and 45.52 % at highest concentration. According to XTT cell viability results, it was observed that NP samples including cisplatin had significantly more effective cytotoxic activity against MDA-MB 231 cells compared to cisplatin alone. Especially, the NP1 formulation showed the highest cytotoxic activity at all concentrations. According to the results of in vitro cell culture studies and XTT cytotoxicity studies, we can conclude that cisplatin has an important antiproliferative effect on MDA-MB 231 cells so has a cytotoxic effect. In addition, the main aim of preparing nanoparticles containing cisplatin in this study was to increase the antiproliferative activity of cisplatin on MDA-MB 231 cells and to obtain a more effective anticancer activity. According to the results, it can be said that nanoparticles show the desired efficiency in XTT cell viability assay.

Table 2. Concentration dependent MDA-MB 231 cell viability results of cisplatin and NPs including cisplatin

Samples/Concentration	MDA-MB 231 Cell Viability (%)				
	(2.5 µg/mL)	(5 µg/mL)	(10 µg/mL)	(25 µg/mL)	(50 µg/mL)
Cisplatin	74.36	71.12	63.74	57.78	47.28
NP1	64.28	61.35	54.28	49.57	41.23
NP2	68.87	63.37	57.58	51.36	43.36
NP3	69.22	64.12	57.86	54.78	45.52

In a study performed Alp et al. HeLa cells were treated with different concentrations of CDDP at 24 and 48 hours. The XTT technique was used to measure cell viability. Furthermore, using Real-Time PCR, the quantitative mRNA expression of the mTOR, AKT, CCND1, and STAT-3 genes was examined following treatment with various dosages of cisplatin. In summary, different mRNA expression pattern was found after CDDP treatment regarding to exposure time [25].

In another study performed Arslan et al. A549 and SK-MES-1 which had been kept as frozen form in the liquid nitrogen tank were prepared in order to use. The cytotoxicity was determined using XTT and MTT, and the apoptotic effects were assessed first by comparing the mRNA levels of the BAX, BCL-2, and CASPASE-3 genes, and then by counting the percentage of early apoptotic cells observed on the cell lines. When all of the data was analyzed properly, the effects of -Bgtx treatment with and without cisplatin were shown to be significant, particularly in the SK-MES-1 squamous lung cancer line, where tobacco use was the primary cause of cancer with a high percentage of cases. [26].

42 female patients were included in this study who were neoadjuvantly treated with the ETC combination (epirubicin 60 mg/m<sup>2</sup>, docetaxel 60 mg/m<sup>2</sup>, and cisplatin 60 mg/m<sup>2</sup>) every three weeks between March 2010 and March 2011 provided having the diagnosis of breast cancer. There wasn't any treatment related death. Combination of ETC chemotherapy can be preferred in neoadjuvant treatment of breast cancer because of high response rate and tolerability [27]. Unlike our study, cisplatin was used in combination. Epirubicin and docetaxal were used in combination.

Perez et al. reported a high response rate of up to 62% in a study they conducted with paclitaxel and carboplatin as the first choice treatment for patients with metastatic breast cancer. Based on the findings of these research, platinum compounds are becoming more popular as first-line treatments, particularly for triple-negative breast cancer. Cisplatin is utilized alone or in various combinations in these recent research. The inclusion of gemcitabine or capecitabine to anthracycline and taxanes-containing regimens is also being investigated [28].

The advantage of our study over other studies is that we used chitosan polymer in addition to cisplatin. Among

the features of chitosan; Biodegradable, biocompatible, antimicrobial activity, non-toxic, chemical and physical properties, can be converted into a wide variety of physical forms with appropriate technological methods. Since chitosan in cationic structure contains amino group that can react, it can easily react with ions in anionic structure. Chitosan has many advantages, especially with its ability to transform into microspheres, microparticles and nanoparticles. These forms can control the controlled release of active substances. Due to the biocompatible chitosan nanoparticle, it shows antiproliferative activity in cancer cells and prevents the progression of cancer cells. At the same time, it does not have any side effects and toxicity to healthy cells.

## Conclusion

The experiment was to investigate what kind of effect cisplatin has on breast cancer cells by observing its antiproliferative activity. It was aimed to prepare cisplatin-loaded chitosan nanoparticles and obtain better cytotoxic activity on cancer cells. In this study, nanoparticles with positive zeta potential showed the desired effect because they can easily adhere and pass through the cell membrane. The acceptable value of the polydisperse index value should be at most 0.4, and as a result of the experiment, it was found to be less than 0.4. Based on the results of the study, we can conclude that cisplatin loaded nanoparticle has a significant antiproliferative effect on MDA-MB 231 cells.

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## Conflicts of interest

The authors state that did not have conflict of interest.

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