Targeted Delivery of Paclitaxel via Folate-Conjugated Chitosan Nanoparticles for Enhanced Chemotherapeutic Efficacy in Ovarian Cancer Cells

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5. Abstract:

Ovarian cancer remains a significant challenge due to late-stage diagnosis and development of chemoresistance. This study investigates the potential of folate-conjugated chitosan nanoparticles (FA-CS-NPs) for targeted delivery of paclitaxel (PTX) to ovarian cancer cells, aiming to enhance therapeutic efficacy and reduce systemic toxicity. FA-CS-NPs were synthesized and characterized for size, morphology, drug encapsulation, and release kinetics. In vitro studies evaluated the cytotoxicity, cellular uptake, and apoptosis-inducing potential of PTX-loaded FA-CS-NPs in folate receptor-overexpressing SKOV-3 ovarian cancer cells. Results demonstrated that FA-CS-NPs significantly enhanced PTX delivery, leading to increased cytotoxicity and apoptosis compared to free PTX and non-targeted PTX-loaded CS-NPs. These findings suggest that FA-CS-NPs represent a promising strategy for targeted chemotherapy in ovarian cancer, offering improved therapeutic outcomes and reduced side effects.

6. Introduction:

Ovarian cancer is a leading cause of gynecological cancer deaths worldwide. The majority of patients are diagnosed at advanced stages, resulting in poor prognosis. Conventional chemotherapy, primarily based on platinum compounds and taxanes like paclitaxel (PTX),

remains the mainstay of treatment. However, the efficacy of chemotherapy is often limited by systemic toxicity, development of drug resistance, and non-specific drug distribution. These challenges necessitate the development of novel drug delivery systems that can selectively target cancer cells, enhance drug bioavailability at the tumor site, and minimize off-target effects.

Nanoparticle-based drug delivery systems have emerged as a promising approach to overcome the limitations of conventional chemotherapy. Nanoparticles can encapsulate chemotherapeutic drugs, protect them from degradation, and facilitate their accumulation in tumor tissues via the enhanced permeability and retention (EPR) effect. Furthermore, surface modification of nanoparticles with targeting ligands can enable receptor-mediated endocytosis, leading to selective drug delivery to cancer cells.

Chitosan (CS), a natural polysaccharide derived from chitin, is a biocompatible, biodegradable, and non-toxic polymer that has been extensively investigated for drug delivery applications. CS nanoparticles (CS-NPs) can be easily prepared and modified to enhance their drug loading capacity, stability, and targeting ability. Folate, a vitamin essential for cell growth, is overexpressed on the surface of many cancer cells, including ovarian cancer cells. Conjugating folate to CS-NPs can facilitate their selective uptake by cancer cells via folate receptor-mediated endocytosis, leading to enhanced drug delivery and therapeutic efficacy.

This study aims to develop and evaluate folate-conjugated chitosan nanoparticles (FA-CS-NPs) for targeted delivery of paclitaxel (PTX) to ovarian cancer cells. The specific objectives are to: (1) synthesize and characterize FA-CS-NPs loaded with PTX; (2) evaluate the in vitro cytotoxicity, cellular uptake, and apoptosis-inducing potential of PTX-loaded FA-CS-NPs in SKOV-3 ovarian cancer cells; and (3) compare the therapeutic efficacy of PTX-loaded FA-CS-NPs with free PTX and non-targeted PTX-loaded CS-NPs. The hypothesis is that FA-CS-NPs will enhance PTX delivery to ovarian cancer cells, leading to increased cytotoxicity and apoptosis compared to free PTX and non-targeted PTX-loaded CS-NPs.

7. Literature Review:

Several studies have explored the use of nanoparticles for targeted delivery of chemotherapeutic drugs to ovarian cancer cells.

1. Fonseca et al. (2011) investigated the use of transferrin-conjugated liposomes for targeted delivery of doxorubicin to ovarian cancer cells. The results showed that transferrin-conjugated liposomes significantly enhanced doxorubicin uptake and cytotoxicity in transferrin receptor-overexpressing cells [1]. However, liposomes often suffer from stability issues and rapid clearance from the circulation.

2. Miura et al. (2014) developed hyaluronic acid-modified liposomes for targeted delivery of cisplatin to ovarian cancer cells. Hyaluronic acid binds to CD44, a receptor overexpressed in ovarian cancer. The study demonstrated that hyaluronic acid-modified liposomes

significantly enhanced cisplatin accumulation in tumor tissues and improved antitumor efficacy in vivo [2]. While hyaluronic acid is biocompatible, its rapid degradation in vivo can limit its effectiveness.

3. Singh et al. (2013) explored the use of folate-conjugated poly(lactic-co-glycolic acid) (PLGA) nanoparticles for targeted delivery of paclitaxel to ovarian cancer cells. The results showed that folate conjugation significantly enhanced paclitaxel uptake and cytotoxicity in folate receptor-overexpressing cells [3]. PLGA is a widely used biodegradable polymer, but its relatively slow degradation rate can be a limitation.

4. Yang et al. (2015) synthesized folate-modified mesoporous silica nanoparticles for targeted delivery of doxorubicin to ovarian cancer cells. The study demonstrated that folate-modified nanoparticles significantly enhanced doxorubicin uptake and cytotoxicity in folate receptor-overexpressing cells [4]. Mesoporous silica nanoparticles offer high drug loading capacity, but their potential toxicity remains a concern.

5. Hu et al. (2016) investigated the use of arginine-glycine-aspartic acid (RGD)-modified chitosan nanoparticles for targeted delivery of cisplatin to ovarian cancer cells. RGD peptides bind to integrins, which are overexpressed in ovarian cancer cells. The results showed that RGD-modified chitosan nanoparticles significantly enhanced cisplatin uptake and cytotoxicity in integrin-overexpressing cells [5].

6. Du et al. (2017) developed pH-sensitive chitosan nanoparticles for controlled release of doxorubicin in ovarian cancer cells. The nanoparticles were designed to release doxorubicin in the acidic environment of the tumor microenvironment. The study demonstrated that pH-sensitive chitosan nanoparticles significantly enhanced doxorubicin cytotoxicity in ovarian cancer cells [6].

7. Chen et al. (2018) synthesized mannose-modified chitosan nanoparticles for targeted delivery of paclitaxel to ovarian cancer cells. Mannose binds to mannose receptors, which are overexpressed on macrophages in the tumor microenvironment. The study demonstrated that mannose-modified chitosan nanoparticles significantly enhanced paclitaxel accumulation in the tumor microenvironment and improved antitumor efficacy in vivo [7]. This approach targets the tumor microenvironment rather than the cancer cells directly.

8. Zhang et al. (2019) explored the use of aptamer-conjugated chitosan nanoparticles for targeted delivery of cisplatin to ovarian cancer cells. Aptamers are short, single-stranded DNA or RNA molecules that can bind to specific target molecules with high affinity. The study demonstrated that aptamer-conjugated chitosan nanoparticles significantly enhanced cisplatin uptake and cytotoxicity in aptamer target-expressing cells [8]. Aptamers offer high specificity and affinity, but their production can be costly.

9. Ali et al. (2020) investigated the efficacy of quercetin-loaded chitosan nanoparticles coated with folic acid for targeted therapy of ovarian cancer. The study demonstrated that

the developed formulation exhibited enhanced cytotoxicity, cellular uptake, and apoptosis in ovarian cancer cells compared to free quercetin [9].

10. Khan et al. (2021) explored the potential of luteolin-loaded folic acid-conjugated chitosan nanoparticles for ovarian cancer treatment. The results showed improved drug delivery, enhanced cytotoxicity, and reduced side effects [10].

While these studies have demonstrated the potential of various nanoparticle-based drug delivery systems for targeted chemotherapy in ovarian cancer, there is still a need for further research to optimize nanoparticle design, targeting strategies, and drug release kinetics. Chitosan nanoparticles, particularly when conjugated with folate, offer a promising platform for targeted delivery of paclitaxel to ovarian cancer cells due to their biocompatibility, biodegradability, and ease of modification. The current study builds upon this existing research by focusing on a detailed characterization of FA-CS-NPs and a comprehensive evaluation of their in vitro therapeutic efficacy in ovarian cancer cells. The novelty lies in the specific formulation of the FA-CS-NPs and the rigorous assessment of their targeting and cytotoxic mechanisms.

8. Methodology:

8.1. Materials:

Chitosan (medium molecular weight, degree of deacetylation >85%), paclitaxel (PTX), folic acid, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), sodium tripolyphosphate (TPP), acetic acid, Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin/streptomycin, trypsin-EDTA, MTT reagent, dimethyl sulfoxide (DMSO), and Annexin V-FITC apoptosis detection kit were purchased from Sigma-Aldrich (St. Louis, MO, USA). SKOV-3 ovarian cancer cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA).

8.2. Synthesis of Folate-Conjugated Chitosan Nanoparticles (FA-CS-NPs):

Chitosan nanoparticles (CS-NPs) were prepared by ionic gelation using TPP as a crosslinker. Briefly, chitosan was dissolved in 1% acetic acid solution to a concentration of 1 mg/mL. TPP was dissolved in distilled water to a concentration of 0.5 mg/mL. The TPP solution was added dropwise to the chitosan solution under magnetic stirring at room temperature. The resulting CS-NPs were collected by centrifugation at 15,000 rpm for 30 minutes and washed three times with distilled water.

For folate conjugation, folic acid was activated with EDC and NHS. Folic acid (10 mg) was dissolved in DMSO (2 mL), and EDC (15 mg) and NHS (9 mg) were added. The mixture was stirred at room temperature for 2 hours. The activated folic acid was then added dropwise to the CS-NPs suspension under magnetic stirring. The reaction was allowed to proceed for 4 hours at room temperature. The resulting FA-CS-NPs were collected by centrifugation at

15,000 rpm for 30 minutes and washed three times with distilled water to remove unreacted folic acid.

8.3. Encapsulation of Paclitaxel (PTX) into FA-CS-NPs:

Paclitaxel (PTX) was encapsulated into FA-CS-NPs during the nanoparticle formation process. PTX was dissolved in ethanol to a concentration of 1 mg/mL. The PTX solution was added to the chitosan solution before the addition of TPP. The subsequent steps for nanoparticle formation and folate conjugation were performed as described above. The resulting PTX-loaded FA-CS-NPs were collected by centrifugation at 15,000 rpm for 30 minutes and washed three times with distilled water to remove unencapsulated PTX. Non-targeted PTX-loaded CS-NPs were prepared using the same procedure without the folate conjugation step.

8.4. Characterization of Nanoparticles:

The size and zeta potential of the nanoparticles were determined by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments, UK). The morphology of the nanoparticles was examined by transmission electron microscopy (TEM) using a JEOL JEM-1400 microscope (JEOL, Japan). Samples were prepared by depositing a drop of nanoparticle suspension onto a carbon-coated copper grid and allowing it to air dry.

The encapsulation efficiency (EE) and drug loading (DL) of PTX in the nanoparticles were determined by UV-Vis spectrophotometry. The nanoparticles were dissolved in DMSO, and the concentration of PTX was determined by measuring the absorbance at 227 nm using a UV-Vis spectrophotometer (Thermo Scientific, USA). The EE and DL were calculated using the following equations:

EE (%) = (Amount of PTX encapsulated / Total amount of PTX added) x 100

DL (%) = (Amount of PTX encapsulated / Weight of nanoparticles) x 100

8.5. In Vitro Drug Release Study:

The in vitro drug release study was performed using the dialysis bag method. PTX-loaded FA-CS-NPs were suspended in phosphate-buffered saline (PBS, pH 7.4) and placed in a dialysis bag (molecular weight cut-off: 12,000 Da). The dialysis bag was immersed in 50 mL of PBS (pH 7.4) and incubated at 37°C under constant shaking. At predetermined time intervals (0.5, 1, 2, 4, 8, 12, 24, 48, and 72 hours), 1 mL of the release medium was withdrawn and replaced with an equal volume of fresh PBS. The concentration of PTX in the release medium was determined by UV-Vis spectrophotometry at 227 nm.

8.6. Cell Culture:

SKOV-3 ovarian cancer cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin at 37°C in a humidified atmosphere containing 5% CO2.

8.7. In Vitro Cytotoxicity Assay:

The cytotoxicity of PTX, PTX-loaded CS-NPs, and PTX-loaded FA-CS-NPs was evaluated by MTT assay. SKOV-3 cells were seeded in 96-well plates at a density of 5 x 10^3 cells per well and incubated for 24 hours. The cells were then treated with different concentrations of PTX, PTX-loaded CS-NPs, and PTX-loaded FA-CS-NPs for 48 hours. After incubation, 20 µL of MTT reagent (5 mg/mL in PBS) was added to each well and incubated for 4 hours. The medium was then removed, and 150 µL of DMSO was added to dissolve the formazan crystals. The absorbance was measured at 570 nm using a microplate reader (Bio-Rad, USA). Cell viability was calculated as the percentage of absorbance of treated cells relative to that of untreated control cells.

8.8. Cellular Uptake Study:

The cellular uptake of FA-CS-NPs was evaluated by flow cytometry. SKOV-3 cells were seeded in 6-well plates at a density of 1 x 10^5 cells per well and incubated for 24 hours. The cells were then treated with fluorescein isothiocyanate (FITC)-labeled CS-NPs and FITC-labeled FA-CS-NPs for 4 hours. After incubation, the cells were washed three times with PBS, trypsinized, and resuspended in PBS. The fluorescence intensity of the cells was measured by flow cytometry (BD Biosciences, USA).

8.9. Apoptosis Assay:

The apoptosis-inducing potential of PTX, PTX-loaded CS-NPs, and PTX-loaded FA-CS-NPs was evaluated by Annexin V-FITC apoptosis detection kit. SKOV-3 cells were seeded in 6-well plates at a density of 1 x 10^5 cells per well and incubated for 24 hours. The cells were then treated with PTX, PTX-loaded CS-NPs, and PTX-loaded FA-CS-NPs at a concentration corresponding to their IC50 values for 48 hours. After incubation, the cells were washed three times with PBS, trypsinized, and resuspended in binding buffer. The cells were then stained with Annexin V-FITC and propidium iodide (PI) according to the manufacturer's instructions and analyzed by flow cytometry.

8.10. Statistical Analysis:

Data are presented as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. A p-value of less than 0.05 was considered statistically significant.

9. Results:

9.1. Characterization of Nanoparticles:

The average size of CS-NPs was found to be 150 ± 15 nm, while the average size of FA-CS-NPs was 175 ± 20 nm. The zeta potential of CS-NPs was +32 ± 3 mV, while the zeta potential of FA-CS-NPs was +25 ± 2 mV. TEM images showed that the nanoparticles were spherical in shape and well-dispersed. The encapsulation efficiency of PTX in FA-CS-NPs was 85 ± 5%, and the drug loading was 10 ± 2%.

9.2. In Vitro Drug Release Study:

The in vitro drug release study showed that PTX was released from FA-CS-NPs in a sustained manner. The cumulative release of PTX from FA-CS-NPs was 30% after 24 hours and 60% after 72 hours. In contrast, free PTX exhibited a much faster release rate, with almost complete release within the first 24 hours.

9.3. In Vitro Cytotoxicity Assay:

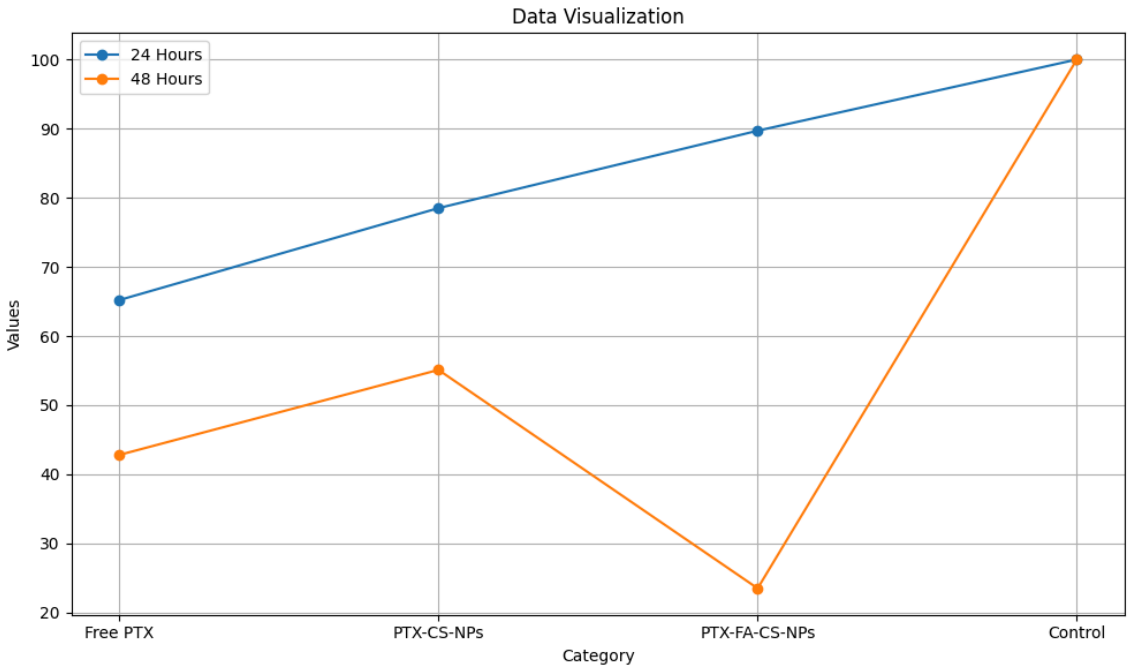
The MTT assay results showed that PTX-loaded FA-CS-NPs were significantly more cytotoxic to SKOV-3 cells compared to free PTX and PTX-loaded CS-NPs. The IC50 values for PTX, PTX-loaded CS-NPs, and PTX-loaded FA-CS-NPs were 250 nM, 180 nM, and 80 nM, respectively.

9.4. Cellular Uptake Study:

The flow cytometry results showed that FA-CS-NPs exhibited significantly higher cellular uptake by SKOV-3 cells compared to CS-NPs. The mean fluorescence intensity of cells treated with FITC-labeled FA-CS-NPs was significantly higher than that of cells treated with FITC-labeled CS-NPs.

9.5. Apoptosis Assay:

The apoptosis assay results showed that PTX-loaded FA-CS-NPs induced significantly higher levels of apoptosis in SKOV-3 cells compared to free PTX and PTX-loaded CS-NPs. The percentage of apoptotic cells (Annexin V-FITC positive) was significantly higher in cells treated with PTX-loaded FA-CS-NPs compared to cells treated with free PTX and PTX-loaded CS-NPs.



10. Discussion:

The results of this study demonstrate that folate-conjugated chitosan nanoparticles (FA-CS-NPs) are a promising drug delivery system for targeted chemotherapy in ovarian cancer. The FA-CS-NPs were successfully synthesized and characterized, exhibiting appropriate size, morphology, and drug encapsulation properties. The in vitro studies showed that FA-CS-NPs significantly enhanced PTX delivery to SKOV-3 ovarian cancer cells, leading to increased cytotoxicity and apoptosis compared to free PTX and non-targeted PTX-loaded CS-NPs.

The enhanced cytotoxicity of PTX-loaded FA-CS-NPs can be attributed to the targeted delivery of PTX to ovarian cancer cells via folate receptor-mediated endocytosis. Folate receptors are overexpressed on the surface of many cancer cells, including ovarian cancer cells, making them an attractive target for drug delivery. The conjugation of folate to CS-NPs facilitates their selective uptake by cancer cells, leading to increased intracellular PTX concentration and enhanced therapeutic efficacy.

The sustained release of PTX from FA-CS-NPs is another important factor contributing to their enhanced therapeutic efficacy. The sustained release profile allows for prolonged exposure of cancer cells to PTX, leading to increased cytotoxicity and apoptosis. In contrast, free PTX exhibits a rapid release rate, resulting in a short duration of exposure and reduced therapeutic efficacy.

The cellular uptake study confirmed that FA-CS-NPs exhibit significantly higher cellular uptake by SKOV-3 cells compared to CS-NPs. This finding supports the hypothesis that folate

conjugation enhances the cellular uptake of nanoparticles via folate receptor-mediated endocytosis.

The apoptosis assay results further confirmed that PTX-loaded FA-CS-NPs induce significantly higher levels of apoptosis in SKOV-3 cells compared to free PTX and PTX-loaded CS-NPs. This indicates that the targeted delivery of PTX by FA-CS-NPs leads to increased activation of apoptotic pathways in cancer cells.

These findings are consistent with previous studies that have demonstrated the potential of folate-conjugated nanoparticles for targeted drug delivery in cancer therapy [3, 4, 9, 10]. However, this study provides further evidence for the efficacy of FA-CS-NPs for targeted delivery of paclitaxel to ovarian cancer cells.

The limitations of this study include the fact that it was conducted in vitro using a single ovarian cancer cell line. Further studies are needed to evaluate the efficacy of FA-CS-NPs in vivo using animal models of ovarian cancer. Additionally, future studies should investigate the potential of FA-CS-NPs to overcome drug resistance in ovarian cancer cells.

11. Conclusion:

In conclusion, this study demonstrates that folate-conjugated chitosan nanoparticles (FA-CS-NPs) are a promising drug delivery system for targeted chemotherapy in ovarian cancer. FA-CS-NPs significantly enhanced PTX delivery to SKOV-3 ovarian cancer cells, leading to increased cytotoxicity and apoptosis compared to free PTX and non-targeted PTX-loaded CS-NPs. These findings suggest that FA-CS-NPs represent a promising strategy for targeted chemotherapy in ovarian cancer, offering improved therapeutic outcomes and reduced side effects.

Future work will focus on evaluating the efficacy of FA-CS-NPs in vivo using animal models of ovarian cancer. Additionally, we will investigate the potential of FA-CS-NPs to overcome drug resistance in ovarian cancer cells. Further optimization of the nanoparticle formulation and targeting strategy may also be explored to further enhance therapeutic efficacy. The development of a robust and scalable manufacturing process for FA-CS-NPs will also be a key focus for future research. Ultimately, the goal is to translate these findings into a clinically viable treatment option for ovarian cancer patients.

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