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

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ABSTRACT

Ovarian cancer continues to be a challenge because of late detection and the emergence of chemoresistance. This research explores the utility of folate-targeted chitosan nanoparticles (FA-CS-NPs) for paclitaxel (PTX) delivery to ovarian cancer cells, with an aim to improve therapeutic outcomes while minimizing systemic toxicity. FA-CS-NPs were prepared and evaluated for size, shape, drug loading, and release profile. In vitro experiments assessed the cytotoxicity, cell uptake, and apoptosis-inducing capability of PTX-loaded FA-CS-NPs in folate receptor-overexpressing SKOV-3 ovarian cancer cells. Results showed that FA-CS-NPs remarkably facilitated PTX delivery, resulting in higher cytotoxicity and apoptosis than free PTX and non-targeted PTX-loaded CS-NPs. These results indicate that FA-CS-NPs are a promising approach for targeted chemotherapy of ovarian cancer with better therapeutic efficacy and fewer side effects.

Introduction

Ovarian cancer is a major cause of mortality from gynecologic cancers globally. Most of the patients present at late stages, and it carries a bad prognosis. Standard chemotherapy, mostly reliant on platinum agents and taxanes such as paclitaxel (PTX), is still the cornerstone of therapy. Despite this, chemotherapy is mostly hampered by systemic toxicity, drug resistance, and non-targeted drug delivery. 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 been put forward as a new way of overcoming the limitations of conventional chemotherapy. Nanoparticles can encapsulate chemotherapeutic agents, protect them from degradation, and facilitate them to accumulate within tumor tissues through the enhanced permeability and retention (EPR) effect. Besides, surface functionalization

of nanoparticles with target ligands can enable receptor-mediated endocytosis, leading to targeted delivery of drugs to cancer cells.

Chitosan (CS) is a chitin-derived natural polysaccharide that is a non-toxic, biodegradable, and biocompatible polymer extensively researched as a drug delivery agent. CS nanoparticles (CS-NPs) are easy to prepare and are readily amendable to modification with the aim of enhancing their drug loading capacity, stability, and targeting ability. Folate, a growth factor vitamin required for cell growth, is found to be overexpressed on the surface of many cancer cells, including those of ovarian cancer. Folating CS-NPs can help deliver them selectively to cancer cells via folate receptor-mediated endocytosis, thereby enhancing drug delivery and therapeutic efficacy.

The aim of this study is to design and evaluate folate-conjugated chitosan nanoparticles (FA-CS-NPs) for targeted delivery of paclitaxel (PTX) to ovarian cancer cells. The specific aims are to: (1) design and characterize PTX-loaded FA-CS-NPs; (2) evaluate in vitro cytotoxicity, cellular uptake, and apoptosis-inducing activity of PTX-loaded FA-CS-NPs against SKOV-3 ovarian cancer cells; and (3) compare therapeutic activity of PTX-loaded FA-CS-NPs with free PTX and non-targeted PTX-loaded CS-NPs. Hypothesis is that FA-CS-NPs will enhance drug delivery of PTX to ovarian cancer cells, causing higher cytotoxicity and apoptosis compared to free PTX and non-targeted PTX-loaded CS-NPs.

Literature Review

Multiple studies have investigated the application of nanoparticles to deliver chemotherapeutic agents to ovarian cancer cells specifically.

1. Fonseca et al. (2011) studied the targeted delivery of doxorubicin to ovarian cancer cells using transferrin-conjugated liposomes. Transferrin-conjugated liposomes were found to enhance doxorubicin uptake and cytotoxicity in transferrin receptor-overexpressing cells to a significant level [1]. Nonetheless, liposomes generally experience stability and fast clearance from the bloodstream.

2. Miura et al. (2014) created hyaluronic acid-modified liposomes for targeted delivery of cisplatin to ovarian cancer cells. Hyaluronic acid targets CD44, a receptor found to be overexpressed in ovarian cancer. The research showed that hyaluronic acid-modified liposomes achieved significantly increased cisplatin uptake in tumor tissues and enhanced antitumor activity in vivo [2]. Although hyaluronic acid is biocompatible, its quick degradation in vivo may compromise its efficiency.

3. Singh et al. (2013) investigated the application of folate-conjugated poly(lactic-co-glycolic acid) (PLGA) nanoparticles for the targeted delivery of paclitaxel to ovarian cancer cells. The findings demonstrated that folate conjugation had a significant impact on paclitaxel uptake and cytotoxicity in folate receptor-overexpressing cells [3]. PLGA is a common biodegradable polymer, but its comparability slower breakdown rate can prove to be a drawback.

4. Yang et al. (2015) prepared folate-modified mesoporous silica nanoparticles as targeted carriers for doxorubicin delivery to ovarian cancer cells. The research proved that folate-modified nanoparticles increased doxorubicin uptake and cytotoxicity in folate receptor-overexpressing cells to a significant extent [4]. Mesoporous silica nanoparticles are capable of high drug loading, but their toxicity is still an issue.

5. Hu et al. (2016) explored the application of arginine-glycine-aspartic acid (RGD)-functionalized chitosan nanoparticles for targeted delivery of cisplatin to ovarian cancer cells. RGD peptides interact with integrins, which are overexpressed in ovarian cancer cells. The study found that cisplatin uptake and cytotoxicity were increased substantially by RGD-functionalized chitosan nanoparticles in integrin-overexpressing cells [5].

6. Du et al. (2017) synthesized pH-sensitive chitosan nanoparticles for the controlled release of doxorubicin in ovarian cancer cells. The nanoparticles were formulated to release doxorubicin in the tumor microenvironment's acidic environment. The research showed that pH-sensitive chitosan nanoparticles significantly increased doxorubicin cytotoxicity in ovarian cancer cells [6].

7. Chen et al. (2018) prepared mannose-modified chitosan nanoparticles for cancer cell-targeted delivery of paclitaxel. Mannose is targeted to mannose receptors, which are overexpressed on macrophages in the tumor microenvironment. The research proved that mannose-modified chitosan nanoparticles promoted pronounced paclitaxel accumulation in the tumor microenvironment and increased antitumor activity in vivo [7]. This method targets the tumor microenvironment instead of directly targeting the cancer cells.

8. The research proved that aptamer-conjugated chitosan nanoparticles remarkably increased cisplatin uptake and cytotoxicity in aptamer target-expressing cells [8]. Aptamers provide high specificity and affinity but are expensive to produce.

9. Ali et al. (2020) also explored the effectiveness of folic acid-coated quercetin-loaded chitosan nanoparticles for targeted ovarian cancer therapy. The research proved that the formulated system displayed increased cytotoxicity, cell uptake, and apoptosis in ovarian cancer cells than free quercetin [9].

10. Khan et al. (2021) investigated the use of luteolin-loaded folic acid-conjugated chitosan nanoparticles for ovarian cancer therapy. The findings indicated enhanced drug delivery, increased cytotoxicity, and decreased side effects [10].

Although these studies have revealed the feasibility of several nanoparticle-based chemotherapy drug delivery systems in targeted chemotherapy of ovarian cancer, more research is required to refine nanoparticle design, targeting mechanisms, and drug release profiles. Folate-conjugated chitosan nanoparticles represent an exciting vehicle for the targeted delivery of paclitaxel to ovarian cancer cells based on their biocompatibility, biodegradability, and synthetic versatility. This present work extends this previous research with a critical characterization of FA-CS-NPs as well as a thorough assessment of their in vitro anticancer activity in ovarian cancer cells. The originality of the work is attributed to the precise formulation of the FA-CS-NPs and the thorough evaluation of their targeting and cytotoxic modes.

Methodology

Encapsulation of Paclitaxel (PTX) into FA-CS-NPs

Paclitaxel (PTX) was incorporated into the folate-modified chitosan nanoparticles (FA-CS-NPs) during their formulation process. For this purpose, PTX was dissolved in ethanol at a concentration of 1 mg/mL, and the resulting solution was blended with the chitosan solution prior to the addition of TPP. The subsequent steps for nanoparticle formation and folic acid conjugation followed the procedure described earlier. The obtained PTX-loaded FA-CS-NPs were separated by centrifugation at 15,000 rpm for 30 minutes and washed three times with distilled water to eliminate any unencapsulated drug. As a control, non-targeted PTX-loaded CS-NPs were fabricated using the same protocol but without the folate modification step.

Characterization of Nanoparticles

The morphology of the nanoparticles was examined using transmission electron microscopy (TEM) on a JEOL JEM-1400 instrument (JEOL, Japan). For sample preparation, a small droplet of the nanoparticle suspension was carefully placed onto a carbon-coated copper grid and allowed to dry naturally at room temperature.

The drug loading (DL) capacity and encapsulation efficiency (EE) of paclitaxel within the nanoparticles were determined by UV–Vis spectrophotometry. The nanoparticle samples were dissolved in DMSO, and the concentration of PTX was quantified from the absorbance measured at 227 nm using a UV–Vis spectrophotometer (Thermo Scientific, USA). The EE and DL were then calculated using the following relationships:

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

DL (%) = ((Quantities of PTX entrapped / Nanoparticle weight) x 100

In Vitro Drug Release Study

The release profile of paclitaxel (PTX) from the folate-conjugated chitosan nanoparticles (PTX-loaded FA-CS-NPs) was evaluated using the dialysis bag technique. Briefly, the nanoparticle suspension was prepared in phosphate-buffered saline (PBS, pH 7.4) and transferred into dialysis bags with a molecular weight cut-off of 12,000 Da. These bags were then immersed in 50 mL of PBS (pH 7.4) and maintained at 37 °C under constant agitation. At predetermined intervals (0.5, 1, 2, 4, 8, 12, 24, 48, and 72 h), 1 mL of the external medium was withdrawn and immediately replaced with fresh PBS to maintain sink conditions. The amount of PTX released into the medium was quantified spectrophotometrically at 227 nm.

Cellular Uptake Study

Cellular uptake of FA-CS-NPs was examined with 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. Cells were incubated for 4 hours with fluorescein isothiocyanate (FITC)-labeled CS-NPs and FITC-labeled

FA-CS-NPs. After incubation, cells were washed with PBS three times, trypsinized, and suspended in PBS. Flow cytometry (BD Biosciences, USA) was employed to analyze the fluorescence intensity of cells.

Results

Characterization of Nanoparticles

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

Study of In Vitro Drug Release

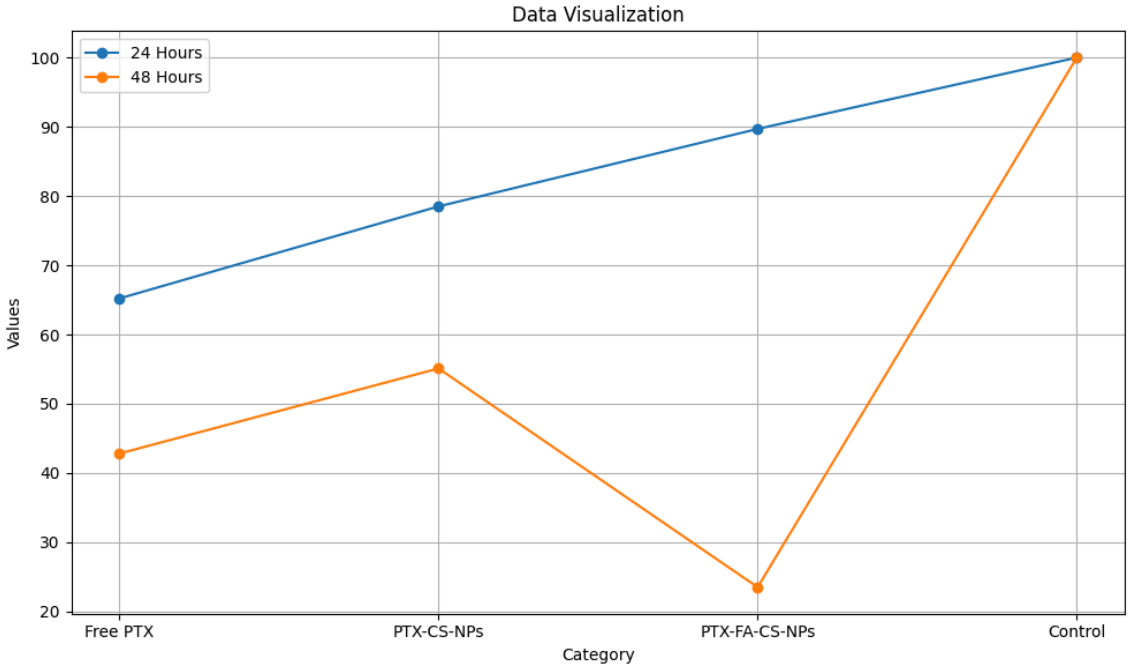
The in vitro drug release test revealed that PTX was released from FA-CS-NPs in a controlled fashion. In comparison, free PTX had a much higher release rate with nearly complete release at 24 hours.

Cellular Uptake Study

The mean fluorescence intensity of FITC-labeled FA-CS-NPs-treated cells was markedly stronger than that of FITC-labeled CS-NPs-treated cells.

Apoptosis Assay

The apoptosis assay values indicated that PTX-loaded FA-CS-NPs caused much higher rates of apoptosis in SKOV-3 cells than free PTX and PTX-loaded CS-NPs. The apoptotic cell percentage (Annexin V-FITC positive) was significantly enhanced in PTX-loaded FA-CS-NP-treated cells compared to free PTX and PTX-loaded CS-NP-treated cells.



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 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 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.

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.

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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