Chitosan Nanoparticles Enhances The Cytotoxic Effects Of Tamoxifen In Breast Cancer Cells

Rajkumari Thagele and Mohan Lal Kori*
Vedica College of B. Pharmacy, RKDF University, Bhopal, Madhya Pradesh,India
mlkori.research@gmail.com
9893968611

Corresponding author

Dr. Mohan Lal Kori

Vedica College of B. Pharmacy, RKDF University, Bhopal, Madhya Pradesh, India

Chitosan Nanoparticles Enhances The Cytotoxic Effects Of Tamoxifen In Breast Cancer Cells

Rajkumari Thagele and Mohan Lal Kori*

Vedica College of B. Pharmacy, RKDF University, Bhopal, Madhya Pradesh,India

mlkori.research@gmail.com

9893968611

Abstract

Objective: This study was aimed at examining an effect of tamoxifen loaded chitosan nanoparticles on growth and proliferation of breast cancer cells.

Background: Breast cancer is one of the most common cancers and the second leading cause of cancer death among women worldwide. Tamoxifen is the most widely used anti-estrogen for the treatment of breast cancer. We hypothesize that Tamoxifen loaded chitosan nanoparticles can showed better result from this anticancer drug.

Methods: Breast cancer cell lines, MCF-7, were treated with developed Tamoxifen loaded chitosan nanoparticles at 24, 48 or 72 hours for MCF-7. We used the MTT assay and lactate dehydrogenase leakage (LDH) assay to evaluate cell viability and cytotoxicity, respectively.

Conclusion: We have demonstrated that Tamoxifen loaded chitosan nanoparticles were internalized well in breast cancer cells in vitro, suggesting their suitability in breast cancer treatment. Preferential uptake of nanoparticles rather than the free drug by MCF-7 cells causes the cells to be more viable to the free drug.

Key words: Breast cancer, Tamoxifen, Chitosan, MTT

Introduction

About one-fifth of cancer patients suffer from breast cancer worldwide.[1]Various chemotherapeutic agents are used to treat the breast cancer. The existing anticancer agents do not greatly differentiate between the cancerous and normal cells, leading to systemic toxicity and adverse effects. This greatly limits the maximum permissible dose of the drug. Drug permeation into the cancer cells from the conventional formulation is very poor due to less distribution and quick elimination. The extensive distribution and rapid elimination from targeted organs result in a greater requirement of the drug by the tissue, which causes undesirable toxicity as well as being economically unsound.[2] Polymeric nanoparticles play an important role in delivering such kinds of chemotherapeutic agents in a controlled manner. Delivering the drugs through the nanoparticles makes it possible to achieve the desired concentration of drug in the specific site, thus minimizing the side effects and reducing the toxicitydose dumping, etc. Depending on the molecular weight and copolymer ratio, the degradation time of chitosan can vary from several

months to years.[3, 6] Different anticancer drugs, including Tamoxifen , were loaded in the chitosan nanoparticles by many researchers.[7–10] Tamoxifen , an antiestrogenic compound, is the first choice for hormonal treatment of breast cancer in both post and premenopausal women for the last few decades. It is often used as an adjuvant therapy following primary treatment of early stage breast cancer. Depending upon the dose and tissue, Tamoxifen can act as an antiestrogenic or as an estrogenic agent. For breast cancer it shows an antiestrogenic effect, and on the uterus it shows an estrogenic effect. Depending upon the dose and the concentration it has several side effects, such as endometrial carcinoma for postmenopausal women. Other side effects include liver cancer, venous thrombosis, pulmonary emboli, and an ocular effect includes retinopathy and corneal opacities.[11–14] To overcome such severe side effects, in our research work we have mainly concentrated on the parenteral sustained release delivery of Tamoxifen in nanoparticles so that they can penetrate in the tumor tissue and can be taken up well by endocytosis into the affected cells.

Materials and methods

Tamoxifen was received as a kind gift sample by Khandelwal Laboratories, Mumbai, chitosan from Central of Institute of fisheries technology, Cochin, and fluorescein isothiocyanate98% (FITC) (HiMedia Laboratories) were used in the study. Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum and tetrazolium dye 3-(4,5-dimers dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich Co. All other chemicals used were of analytical reagent grade.

Experimental Method

Tamoxifen loaded chitosan nanoparticles were prepared according to Calvo et al. 1996 and Sacco *et al*., 2016 with slight modifications based on the ionic gelation of chitosan with TPP anions. Chitosan (CH) solution (2mg/ml) was prepared by dissolving CH in acetic acid solution using magnetic stirrer (Aarson, India) and TPP 1mg/ml was dissolved in distilled water at the concentration of 1.0 mg/ml. Tmx solution (10% w/v) was added to the TPP solution and stirred for 5 mins using a magnetic stirrer. Finally, TPP solution was added dropwise to 4ml of chitosan solution using syringe needle under vigorous magnetic stirring at room temperature leading to formation of Tmx loaded chitosan nanoparticles.Sonicate the formulation for 15 minutes using sonicator (Digital Ultrasonic Cleaner, Jyoti Scientific, Gwalior), then disperse in water and centrifuge for 30 minutes (Rolex Centrifuge) .The supernatant was discarded and sediment was taken and washed three times with distilled water and lyophilized. The lyophilized formulation was used for further characterization.

Cellular uptake study

Confocal laser scanning microscopy was used to visualize the uptake of the polymeric nanoparticles within the cancer cells. For fluorescence imaging of cellular uptake, MCF-7 cells

(at 104 cells/mL) were cultivated for 24 hours on cover slips in six well culture plates (3 mL/well). TNP (NP4) suspensions, 50 µl/mL and 100 µl/mL, were then added to the cell culture medium at a concentration of 300 µg/mL. The cells were washed three times after incubation for 3 hours and then fixed using 4% paraformaldehyde aqueous solution. After fixing for 15 minutes, they were rinsed with phosphate buffered saline (pH 7.4) solution. After that the cover slips were taken out carefully and placed on the slide and air-dried. Finally, they were observed using a confocal laser scanning microscopy system (Andor Spinning Disc Confocal Microscope; Andor Technology Ltd., Belfast, Northern Ireland, UK).[15]

MTT assay for in vitro cell viability studies MCF-7 cells were cultured in DMEM without phenol red and supplemented with 10% fetal bovine serum. The cell culture medium was maintained at 37°C in a humidified incubator containing 5% CO2 atmosphere. Trypsinized confluent cell monolayers were grown (75%–80%) and the cells in the exponentially growing phase were used for cytotoxicity experiments. The cytotoxicity study of TNPs (NP4) was investigated in MCF-7 cells using the MTT assay method.[16] The cytotoxicity of the nanoparticles was determined after 48 hours incubation with MCF-7 cells. To determine the cell cytotoxicity/viability, the cells were plated at a density of 5×103 cells/well (optimal seeding density) in 96 well plates and the plate was kept at 37°C in 5% CO2 atmosphere in a CO2 incubator (Model MCO-15AC; Sanyo Electric Biomedical Co. Ltd., Osaka, Japan). After 12 hours of incubation, the medium in the wells was replaced by a fresh medium containing nanoparticles (added to the medium just before its incorporation in the well) with varying concentrations. After 48 hours, MTT dye solution was added to each well. The incubation was continued for a further 4 hours at 37°C and 5% CO2 for exponentially growing cells. Then the medium in each well containing unbound MTT and death cells was removed by suction. The formazan crystals were solubilized with 100 µL dimethylsulfoxide, and the solution was vigorously mixed to dissolve the reacted dye. All the experiments were performed in triplicate. The absorbance of each well was read on a microplate reader (multimode plate reader, SpectraMax M5; Molecular Devices, CA, USA) at 540 nm. Two different experimental control media, one containing nanoparticles without a drug and the other containing a free drug, were used. The cell viability (%) of the drug containing nanoparticles related to the control wells containing nanoparticles without a drug. and a free drug respectively, was calculated by absorbance of test sample/absorbance of the control sample ×100.

Cellular uptake study

To investigate the cellular uptake of the nanoparticles in MCF-7 cells, a short-term *in vitro* particle endocytosis test was carried out using FITC-TNPs. It shows that Tamoxifen loaded chitosan nanoparticles penetrated the cell membrane and were distributed in the cytoplasm, but not in the nuclei. Moreover, the green fluorescent dots in the samples increased with the increasing concentrations of the nanoparticles. These images have demonstrated concentration dependent endocytosis of nanoparticles in MCF-7 cells.

In vitro cytotoxic assay

The proliferation/viability of MCF-7 cells was assessed by MTT assay after 48 hours of incubation with the free drug and nanoparticles with or without the drug, respectively (Figure 9). The cytotoxic effect of nanoparticles increased with an increase in Tamoxifen concentration. The toxicity of Tamoxifen increased as the drug concentration increased from 12.5 μM to 200 μM. The toxic effect of Tamoxifen is markedly decreased the cell, viability from 70.13% to 21.81%. In this cytotoxicity test, TNPs caused more death of viable cells than Tamoxifen alone (free drug). Again, nanoparticles without the drug failed to produce any toxicity to viable cells and exhibited viability of 99.11%. This increased toxicity may be due to the preferential uptake of nanoparticles than that of the free drug (Tamoxifen).

The cytotoxicity of TNPs was more than that of Tamoxifen alone. The possible mechanism underlying the enhanced efficacy of TNPs against MCF-7 may include the enhanced intracellular drug accumulation by nanoparticle uptake.[17-20] However, the advantage of TNPs over Tamoxifen free drug is that a single dose of Tamoxifen loaded chitosan nanoparticles will provide a much longer drug action (sustained) as compared to a single dose of free drug and may provide passive targeting due to the enhanced permeability and retention effect as reported earlier. Tamoxifen loaded chitosan nanoparticles with positive zeta potentials, which have been reported to be cleared from the blood very quickly as compared to the nanoparticle with negative zeta value. In our study, the prepared nanoparticles had negative zeta potentials which generally allow them to be in the blood circulation for a longer time.

Tamoxifen loaded chitosan nanoparticles along with MTT assay, as investigated here, and the results of the study show concentration dependent cellular uptake of nanoparticle in vitro by MCF-7 breast cancer cells. Drug release also varied predominantly among the studies. Thus the present study has its own uniqueness.

Conclusion

The outcome of the present investigation proposes a novel formulation of Tamoxifen loaded chitosan nanoparticles, prepared by an ionotropic gelation technique. The polymeric particles in a nanosize range with a desired drug polymer ratio can be produced. The size, drug loading and the drug release kinetics can be optimally controlled. Further, Tamoxifen loaded chitosan nanoparticles were internalized well in breast cancer cells in vitro, suggesting their suitability in breast cancer treatment. Preferential uptake of nanoparticles rather than the free drug by MCF-7 cells causes the cells to be more viable to the free drug.

References

1. Malik AA, Wani KA, Ahmad SR. Breast conservative therapy. J Med Sci. 2012; 15(1):7–14.

2. Sinha R, Kim GJ, Nie S, Shin DM. Nanotechnology in cancer therapeutics: bioconjugated nanoparticles for drug delivery. Mol Cancer Ther. 2006; 5(8):1909–1917.

3. Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A, Préat V. PLGA-based nanoparticles: an overview of biomedical applications. J Control Release. 2012; 161(2):505–522.

4. Lü JM, Wang X, Marin-Muller C, et al. Current advances in research and clinical applications of PLGA-based nanotechnology. Expert Rev Mol Diagn. 2009; 9(4):325–341.

5. Kumari A, Yadav SK, Yadav SC. Biodegradable polymeric nanoparticles based drug delivery systems. Colloids Surf B Biointerfaces. 2010; 75(1):1–18.

6. Prokop A, Davidson JM. Nanovehicular intracellular delivery systems. J Pharm Sci. 2008;97(9):3518–3590.

7. Averineni RK, Shavi GV, Gurram AK, et al. PLGA 50:50 nanoparticles of paclitaxel: Development, in vitro anti-tumor activity in BT-549 cells and in vivo evaluation. Bull Mater Sci. 2012; 35(3): 319–326.

8. Hassani SM, Bagheri S. Computational procedure for determining physicochemical properties of doxorubicin-PLGA nanoparticles and daunorubicin-PLGA nanoparticles. Int J Pharm Tech Res. 2012;4(3): 1242–1246.

9. Pradhan R, Poudel BK, Ramasamy T, Choi HG, Yong CS, Kim JO. Docetaxel-loaded polylactic acid-co-glycolic acid nanoparticles: formulation, physicochemical characterization and cytotoxicity studies. J Nanosci Nanotechnol. 2013;13(8):5948–5956.

10. Wohlfart S, Khalansky AS, Gelperina S, et al. Efficient chemotherapy of rat glioblastoma using doxorubicin-loaded PLGA nanoparticles with different stabilizers. PLoS One. 2011;6(5):e19121.

11. The BIG 1-98 Collaborative Group. Letrozole therapy alone or in sequence with tamoxifen in women with breast cancer. N Engl J Med. 2009; 361(8):766–776.

12. Peters-Engl C, Frank W, Danmayr E, Friedl HP, Leodolter S, Medl M. Association between endometrial cancer and tamoxifen treatment of breast cancer. Breast Cancer Res Treat.1999;54(3):255–260.

13. de Lima GR, Facina G, Shida JY, et al. Effects of low dose tamoxifen on normal breast tissue from premenopausal women. Eur J Cancer. 2003; 39(7):891–898.

14. Mirzajani F, Rafati H, Atyabi F. Fabrication of biodegradable poly (d, l-lactide-co-glycolide) nanoparticles containing tamoxifen citrate. Iranian Polymer Journal. 2010; 19(6):437–446.

15. 18. Li X, Tian X, Zhang J, et al. In vitro and in vivo evaluation of folate receptor-targeting amphiphilic copolymer-modified liposomes loaded with docetaxel. Int J Nanomedicine. 2011;6:1167–1184.

16. Fonseca C, Simõesa S, Gaspar R. Paclitaxel-loaded PLGA nanoparticles: preparation, physicochemical characterization and in vitro antitumoral activity. J Control Release. 2002;83(2):273–286

17. Li X, Tian X, Zhang J, et al. In vitro and in vivo evaluation of folate receptor-targeting amphiphilic copolymer-modified liposomes loaded with docetaxel. Int J Nanomedicine. 2011;6:1167–1184.

18. Fonseca C, Simõesa S, Gaspar R. Paclitaxel-loaded PLGA nanoparticles: preparation, physicochemical characterization and in vitro antitumoral activity. J Control Release. 2002;83(2):273–286.

19. Zhang L, Hu Y, Jiang X, Yang C, Lu W, Yang YH. Camptothecin derivativeloaded poly(caprolactone-co-lactide)- b-PEG-b-poly(caprolactoneco-lactide) nanoparticles and their biodistribution in mice. J Control Release. 2004;96(1):135–148.

20. Unger F, Wittmar M, Kissel T. Branched polyesters based on poly [vinyl-3-(dialkylamino) alkylcarbamate-co-vinyl acetate-co-vinyl alcohol]-graft-poly (d, l-lactide-co-glycolide): Effects of polymer structure on cytotoxicity. Biomaterials. 2007;28(9):1610–1619.