International Journal of Biological Macromolecules 97 (2017) 115–122

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Characterization and carboplatin loaded chitosan nanoparticles for the chemotherapy against breast cancer *in vitro* studies

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ARTICLE INFO

Article history:
Received 18 October 2016
Received in revised form 2 December 2016
Accepted 30 December 2016

Keywords:
Chitosan
Nanoparticles
Nanocarboplatin
Carboplatin
Anticancer

ABSTRACT

Aim of the studies to synthesized chitosan nanoparticles by an ionic interaction procedure. The nanoparticles were characterized by physicochemical methods like, DLS, TEM, Surface potential measurements, FT-IR and DSC. The average particle size of chitosan and carboplatin nanoparticles was found to be 277.25 ± 11.37 nm and 289.30 ± 8.15 nm and zeta potential was found to be 31 ± 3.14 mV and 33 ± 2.15 mV respectively with low polydispersity index. The maximum entrapment of carboplatin in nanoparticles was a spherical shape with a positive charge. The maximum encapsulation and loading efficiencies of carboplatin (5 mg/ml) were obtained to be 58.43% and 13.27% respectively. The nanocarboplatin was better blood compatibility as compared to chitosan nanoparticles. Finally, the cytotoxic effects of the carboplatin loaded chitosan nanoparticles were tested *in-vitro* against breast cancer (MCF-7) cell lines. Our studies showed that the chitosan nanoparticles could be used as a promising candidate for drug delivery for the therapeutic treatment of breast cancer.

1. Introduction

Cancer is a complex disease which includes many pathological features of abnormal growth in healthy cells, invasion and metastatization. Uncontrolled cellular growth increases the number of abnormal cells; these cells reach the blood stream and affect healthy tissues giving rise to secondary tumors. Among all cancers, breast cancer is the most common and second leading cause of cancer mortality among women [1,2]. It has been diagnosed 50% worldwide in developed countries [3]. A common pathway for breast cancer is related mortality to the development of metastatic breast cancer [4]. The most common part effect for breast cancer metastases are lymph nodes, lung, liver, bones and brain [5] and may develop at any sites following detection of a primary breast cancer. The mostly use treatment for cancers are surgery, radiotherapy, immunotherapy and chemotherapy [6]. Surgery in additional to chemotherapy has better useful in a number of different types of cancer such as breast cancer, pancreatic cancer, ovarian cancer, testicular cancer and certain lung cancers. Thus, the chemotherapeutic drugs are an attractive strategy for effective anticancer treatment. However, the efficacy of the chemotherapy and the side effects varies among drug to drug. Some drugs may have better efficacy but also serious side effects affecting the quality of living life [7]. During the last several years, various techniques have been developed for the drug targeting in cancer and trial of pro-drug, drug structural modification and carrier mediated drug delivery [8]. Although, all the above strategies may offer some improvement in treatment outcome and, they are also associated with few drawbacks. The invasive techniques enable the entry of certain unnecessary substances into body results in high risk of intracranial infections [9]. The pro-drug approach causes loss of drug activity and stability after modification. Recently, colloidal drug carrier systems, especially nanoparticles have become an emerging field of drug-delivery systems for targeting the drug to the cancer cells *via* cell signaling pathways. Nanoparticles are actually solid colloidal particles ranging in average size from 10 to 1000 nm (usually 50–300 nm) [10] in which the active principle is loaded, dissolved, passively adsorbed or covalently attached to the surface of polymers [11]. Nanocarriers act as a vector and improve intracellular uptake and distribution inside the tumor areas [12]. Among, the several colloidal drug delivery systems, polymeric nanoparticles have received greater attention in breast cancer targeting because they have the ability to deliver a wide range of drugs

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http://dx.doi.org/10.1016/j.ijbiomac.2016.12.090
0141-8130/

to targeting tumor regions of the body for controlled drug release and site-specific drug targeting [13]. Polymeric nanoparticles can be prepared from materials like polysaccharides (chitosan) [14]. Chitosan nanoparticles are versatile among polymeric nanoparticulate systems and were used for biomedical applications [15,16]. It has been demonstrated as a better drug delivery carrier for the synthesis of nanoparticles because of its advantageous properties such as biodegradability, safety, prolonged blood circulation time, non-immunogenicity, non-toxicity, and enhanced drug stability, property for sustained and controlled drug release and biocompatibility for drug targeting at the cellular level [17]. The chitosan nanoparticles possess high drug entrapment capacities. The decay time of chitosan nanoparticles can be altered from days to years by varying the ratio of chitosan and TPP [18]. Carboplatin is a second generation platinum-based anti-tumor drug and acts as an alkylating agent as well as a better chemotherapeutic agent for newly diagnosed malignancies, including breast tumors [19]. The antitumor activity of carboplatin is to be due to its interaction with DNA, where by its $Pt(NH_3)_2$ moiety binds covalently to two adjacent guanines bases. It is this Pt-DNA adducts that are lead to the cell death of the cancer cells *via* apoptosis and necrosis [20]. It is clinically used for the treatment of several cancers like ovarian, lung, liver, breast and other types of cancer. It is water soluble and does not freely across the blood–brain barrier. As a consequence, administration of carboplatin entrapped in chitosan nanoparticle should result in drug targeting within the breast. There is also a strong evidence to observe that carboplatin is an efficient in killing breast cancer cells at the desired concentration that are not toxic to normal cells [21]. Furthermore, breast cancer cells tend to be highly efficient to carboplatin, the concentrations of carboplatin achieved in the cells following intravenous and intra-arterial administration are unlikely to be sufficient to achieve a clinically significant effect.

Our study can be focused to minimize the serious side-effects of the drug and enhanced its efficiency by entrapment into a nanoparticulate system. In this study, we have synthesized chitosan nanoparticles containing an appreciable amount of carboplatin. The carboplatin loaded chitosan nanoparticles were further characterized by DLS, Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM) and differential scanning calorimetry (DSC). In addition, the cytotoxic activity of carboplatin loaded chitosan nanopartcles and native carboplatin were evaluated in a human breast cancer cell line (MCF-7) and also blood compatibility studies. Furthermore, we have also investigated the mechanism of intracellular uptake of these nanoparticles in the breast cancer cell line.

2. Material and methods

2.1. Materials

Chitosan (low molecular weight, degree of deacetylation 75–85%) and glacial acetic acid were all purchased from Sigma-Aldrich chemicals Private Ltd. (Bangalore, India). Sodium tripolyphosphate (TPP) and DMSO were purchased from CDH-laboratory chemical (India). Tissue culture medium, MTT, antibiotic, and trypsin were purchased from Himedia India and Fetal Bovine Serum (FBS) from Gibco, South America. Carboplatin All other chemicals were used of high purity and water also used in experiments was Millipore grade.

2.2. Preparation and synthesis of chitosan and carboplatin loaded nanoparticles

Chitosan nanoparticles were prepared by ionic interaction procedure [22]. Chitosan nanoparticles were synthesized by the mixing 1 mg/ml of (w/v) TPP solution in Millipore water with 2 mg/ml of (w/v) chitosan solution in 1% of acetic acid on magnetic stirrer at room temperature for overnight and then centrifuged at 10,000 × *g*, for 30 min at 4 °C using a (Sigma Centrifuge, USA). The supernatant was discarded and the chitosan nanoparticles pellet was washed with three times in 10% ethanol and finally suspended in 10 ml of 10% ethanol with mild sonication (Toshcon, India) and stored at 4 °C for further studies. Carboplatin (CP) loaded chitosan nanoparticles were prepared by a similar method except for the desired amount of carboplatin was added in a chitosan solution. The entrapment and loading efficiency were measured as described by [23].

2.3. Characterization of chitosan and carboplatin loaded nanoparticles

2.3.1. Particle size and surface potential analysis

The diameters of chitosan and carboplatin nanoparticles were measured by dynamic light scattering (DLS) using (Protein Solutions, Wyatt Technology, Santa Barbara,CA) worked on quasi-elastic light scattering [24]. The surface (Zeta) potential was monitored in the same instrument and method at 25 °C. All measurements were evaluated in triplicates and the average values were taken.

2.3.2. Transmission electron microscopy (TEM)

The internal structure of chitosan and carboplatin nanoparticles were monitored by TEM (Morgagni-26AD: FEI Company, Netherland at All India Institute of Medical Sciences, India) as described earlier [23].

2.3.3. Fourier transforms infrared spectroscopy (FT-IR)

A Fourier transform infrared (FT-IR) spectrum was described by (Shimadzu FTIR 8201-PC) spectrophotometer to analyze the entrapment of carboplatin in the chitosan nanoparticles as described previously [25].

2.3.4. Differential scanning calorimetry (DSC)

The thermal nature of the chitosan, carboplatin, chitosan nanoparticles and craboplatin loaded nanoparticles were evaluated by differential scanning calorimetry (Microcal, USA). An approximate amount of samples (3–6 mg) were weighed and scanned in a temperature range from 0 °C to 210 °C with a heating rate of 15 °C/min per cycle. The inert atmosphere was maintained by purging nitrogen at a rate of 360 cm^3/ min.

2.3.5. Stability studies of nanoparticles

The stability studies of chitosan and carboplatin nanoparticles are going on according to ICH guidelines. Since the nanoparticles were stored at −80◦C for a long time. The sample was withdrawn after 4th month interval and monitored the particle size, surface morphology and drug entrapment.

2.4. In vitro *carboplatin release studies*

The carboplatin release from carboplatin loaded chitosan naoparticles (5 mg) studies was carried out separately at two different pH 5 and pH 7.4 by dissolving of nanoparticles in 10 ml PBS (10 mM) adjusted by 6N HCL and 1N NaOH [26]. The released carboplatin was re-suspended in 1 ml of milliQ water and calculated the absorbance at 208 nm by a UV–vis spectrophotometer (Shimadzu UV 1700-PC, Japan). The supernatant of chitosan nanoparticles was obtained under the same conditions and used as a control. The concentration of carboplatin was evaluated against a calibration curve described from a known amount of carboplatin under similar condition.