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Characterization and anti-proliferative activity of curcumin loaded chitosan nanoparticles in cervical cancer

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ABSTRACT

In the present study the chitosan nanoparticles (CsNPs) and curcumin loaded chitosan nanoparticles (CLCsNPs) were synthesized by tripolyphosphate (TPP) cross-linking method. The nanoparticles were prepared within a zone of appropriate chitosan and TPP concentrations. The average size of CsNPs and CLCsNPs were approximately 189 ± 11.8 nm and 197 ± 16.8 nm, exhibited a zeta potential of +76 ± 5.6 mV and +71 ± 6.4 mV respectively and drug entrapment efficiency was ≈85%. The CLCsNPs and CsNPs were further characterized by different physicochemical methods like transmission electron microscopy (TEM), dynamic light scattering (DLS), HPLC, MALDI-TOF, FT-IR, XRD and UV–vis Spectroscopy. In vitro studies revealed a fast release of ≈35% at pH 5 and ≈25% at pH 7.4 of the drug during the first 3 h, followed by controlled release of curcumin over a period of 120 h and sustained anti-proliferative activity of the drug in a dose and time dependent manner of CLCsNPs and combination with methyl jasmonate. The higher cytotoxicity effect of CLCsNPs may be due to their higher cellular uptake as compared to curcumin. Chitosan nanoparticles were not only stable but also a nontoxic. Our data suggested that curcumin loaded nanoformulations, therefore, might be promising candidates in cancer therapy.

1. Introduction

Cervical cancer is the second most common cancer in women worldwide and the leading cause of cancer mortality and morbidity in developing countries [1,2]. Most cervical cancers contain papillomaviruses (HPV) DNA and express the viral oncogenes E6 and E7 [3,4]. HPV E6 and E7 proteins interact and suppress important cellular regulatory proteins that regulate the function of cell growth, survival, differentiation, apoptosis and genome stability [3–5]. In present scenario, a common method used for the treatment of cancer is chemotherapy, but it is often associated with large number of drawbacks such as, selection of anti-cancer drugs, multidrug resistance, high drug toxicity, undesirable side effect to normal tissue. [6,7]. To this regard, to overcome the drug resistance, side effect, toxicity and increase the therapeutic index is loading of anti-cancer drugs in nanoparticles, which are a new and ideal technology that can greatly overcome the problems related to drug delivery system, retention and penetration through the barrier. Nanoparticles are micron sized polymeric colloidal particles, with diameters ranging from 10 to 500 nm, in which the therapeutic agent of interest can be encapsulated within the polymeric matrix or adsorbed or conjugated onto the surface [7,8].

Chitosan [β-(1–4)-2-amino-2-deoxy-D-glucose], a deacetylated form of chitin, is a naturally occurring linear biodegradable polymer that is made up of N-acetyl-D-glucosamine and d-glucosamine [9]. Therefore, a water soluble formulation with sustained release property is desired for clinical application of curcumin. In this work, curcumin was incorporated into chitosan, a naturally derived polymer possessing inflammatory activity [10] nanoparticles (CLCsNPs) for better stability and controlled release. CSNPs prevent the rapid clearance of drug from site of inflammation to the systemic circulation due tothe vascularity and tissue permeability associated with inflammation [11].

Chitosan nanoparticles (CsNPs) prepared by electrostatic interaction between the cationic chitosan and anionic counter ions sodium tripolyphosphate (TPP) have an ideal as one of the most promising carrier system for drug delivery [12]. The surface charge of CsNPs is tunable by varying the ratio of the two-polymer concentrations and pH sensitive swelling has also been reported [13]. The chitosan nanoparticles prepared using TPP is more stable and do not require any stabilizing or external cross-linking agents.

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Apart from offering an optimum size for particle trafficking into the body, chitosan is also nontoxic, polycationic, biocompatible and biodegradable. The strong muco-adhesive property of chitosan along with its interaction with tight junction facilitates the intracellular transport of hydrophilic macromolecules by opening the tight junction of the mucosal barriers [14–16]. Chitosan based nanoparticle formulations have been used for loading and delivering different drugs and vaccines, like curcumin [17], meningococcal C conjugate [18], diphtheria [19] and tetanus toxoid [20]. Curcumin is a low molecular weight with poor water soluble drug with three active molecules like: 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (curcumin); 1,6-Heptadiene-3,5-dione,1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl) (demethoxy curcumin) and (1E,6E)-1,7-bis(4-hydroxy phenyl) hepta-1,6-diene-3,5-dione (bis-demethoxy curcumin) [17]. Curcuminoids have antioxidant, anti-inflammatory, anti-proliferative, anti-angiogenic [21] anti-tumourogenic, anticoagulant, antibacterial, anticancer, anti-ischemic and wound healing activities [22,23].

In this end, we have focused in the research on curcumin to the development of potential delivery systems to enhance its aqueous solubility, stability and bioavailability as well as controlled delivery of drug near to cancer cells/tissues. Recently, Anand et al. have achieved two fold enhanced in bioavailability of curcumin with PLGA nanoparticles, where as nine time loaded was reported by Shaikh et al. in same polymeric nanoparticles and furthermore, Maiti et al. observed 2.5 fold more bioavailable of curcumin while delivering with phospholipid complex as compared to curcumin [24–26]. In present study, our attention has given for potential delivery systems to increase the drugs bioavailability by enhancing the residential time which subsequently facilitate the absorption of drug through adhesion with the cellular surface. In this view, the best considered strategy to achieve enhanced bioavailability of curcumin is to load it within chitosan nanoparticles (CsNPs).

The aim of this study was to synthesize a chitosan based nanoparticles delivery system that can solubilise curcumin in aqueous environment and clinically relevant, protect it from hydrolytic degradation and delivered curcumin in a controlled manner. In this way, it will improve the bioavailability of delivered curcumin for maximizing tumor therapeutic efficacy and reduce the pharmacokinetic problems. In this regard, the potentiality of the formulated curcumin loaded chitosan nanoparticles was determined by evaluating its reduced size and higher%EE to evaluate in vitro release kinetics, stability, cellular uptake, cytotoxicity and apoptosis inducing activity in cervical cancer cell.

2. Material and methods

2.1. Materials

Chitosan (Cs), Curcumin (C) and Methyl Jasmonate (MJ) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ethylacetate, acetone, ethanol and Sodium tripolyphosphate (TPP), and DMSO were purchased from CDH-laboratory chemical (India). Tissue culture medium, MTT, antibiotic, and trypsin was purchased from Himedia India and FBS from Gibco, South America. All other chemicals were of analytical grade. The water used in all experiments was Millipore grade.

2.2. Preparation of chitosan nanoparticles and curcumin loaded chitosan nanoparticles

Chitosan nanoparticles (CsNPs) were synthesized by an ionic gelation method as described previously by Calvo et al. [12]. The range of the two compounds (positively charged chitosan and negatively charged sodium tripolyphosphate) in different ratio was based on pilot experiments, in which only one type of phenomena was observed: an almost clear solution, an opalescent suspension and no aggregates. The opalescent suspension was of interest to us in the formation of nanoparticles [27]. In brief, an aqueous solution of 9 ml of sodium tripolyphosphate (TPP) (1 mg/ml, pH 5) was added drop wise to 17.5 ml in chitosan solution (1 mg/ml) with and without curcumin (4 mg/ml). The chitosan solution was prepared by dissolving in a 1% acetic acid and the pH was adjusted to 5.0 with 0.1 M NaOH. It was observed that the formation of nanoparticles without any aggregation, even after the particles were left overnight on magnetic stirring. After overnight stirring the (CsNPs) and Curcumin loaded chitosan nanoparticles (CLCsNPs) were collected by centrifugation at 8000 rpm at 4 °C for 30 min (Sigma Centrifuge, USA) and the pellet was washed three times with 10% aqueous ethanol. The pellets were then resuspended in 10% aqueous ethanol and then stored at 4 °C. This formulation was selected for further studies and characterization as well as for entrapment of curcumin.

2.3. Particles size and zeta potential analysis

Particle size and polydispersity index were determined by DynaPro-TC–04 dynamic light scattering equipment (Protein Solutions, Wyatt Technology, Santa Barbara, CA) worked on quasi-elastic light scattering [28]. Briefly, 1 mg/ml of CsNPs and CLCsNPs were prepared in milliQ water and sonicated for 25 s in an ice bath (Toshcon, India). Zeta potential was measured in the same instrument at 25 °C using the same procedure. All measurements were performed in triplicates and the average value was taken.

2.4. Transmission electron microscopy

The internal structure of CsNPS and CLCsNPs was evaluated by TEM (Morgagni-26AD: FEI Company, Netherland at All India Institute of Medical Sciences, India). Samples of CsNPs and CLCsNPs were placed on carbon-coated copper grids and after drying the samples were negatively stained with 1% w/v uranyl acetate for 15 min and wait for air-dry and visualized at 120 kV under microscope. The TEM image capture and analysis were done using Soft Imaging Viewer software for further analysis.

2.5. Characterization of curcumin bound nanoparticles

The Curcumin was extracted from curcumin loaded chitosan nanoparticles and characterized through High Performance Liquid Chromatography (HPLC) [29]. As per protocol, the curcumin loaded chitosan nanoparticles was dissolved in ethanol (1 mg/ml) to rupture its structure. The sample was then subjected to sonication for 30 s interval up to 3 min at 55W (Toshcon, India) followed by centrifugation at 13000 rpm for 15 min at 25 °C (Sigma, USA) to collect a clear supernatant. The obtained supernatant was analyzed by RP-HPLC (Sigma, St. Louis, USA). For this, 5 ml of the sample was injected manually and analyzed in the mobile phase consisting a ratio mixture of 3:2 (v/v) acetonitrile and citric buffer [1% (w/v)] citric acid solution adjusted to pH 3.0 using 0.1N NaoH with a flow rate of 1 ml/min with a C-18 column Macherey-Nagel, Germany, (250 mm × 4.6 mm, 5 μm diameter). The curcumin peak levels were monitored by UV detection at 430 nm. The curcumin extracted from the CLCsNPs in the sample was evaluated by the peak area correlated with the standard curcumin peak at the same retention time.

2.6. MALDI-TOFMS studies

The entrapment of curcumin in chitosan nanoparticles was taken by dissolving the samples in deionized double distilled water