Journal of Drug Delivery Science and Technology 32 (2016) 1–9

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

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

Research paper

Pravastatin-loaded chitosan nanoparticles: Formulation, characterization and cytotoxicity studies

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ARTICLE INFO

Article history:
Received 1 October 2015
Received in revised form
28 November 2015
Accepted 8 January 2016
Available online 11 January 2016

Keywords:
Chitosan nanoparticles
Pravastatin
Characterization
In vitro release
Cytotoxicity

ABSTRACT

Pravastatin (PRV) loaded Chitosan nanoparticles (PRV/CSNPs) were employed as a novel carriers for liver cancer treatment. These nanoparticles were prepared by an ionic gelation method and characterized by FTIR and XRD. The prepared nanoparticles showed the spherical shape of nanoparticles having an average size of 129.8 ± 10.5–270.4 ± 23.3 nm, PDI in the range of 0.238 ± 0.03–0.452 ± 0.05 and zeta potential between 25.1 ± 2.6 and 33.5 ± 2.7 mV. The PRV entrapment efficiency of CSNPs was in the range of 49.05–72.04%. The in vitro release studies showed an initial rapid PRV release up to 6 h followed by a slow release ranging from 52 to 92% after 48 h following Higuchi's model kinetics. The in vitro cytotoxicity of PRV/CSNPs showed 51% HepG2 growth inhibition compared to 38% of free PRV after 72 h incubation. PRV/CSNPs can be considered as a promising carrier for cancer therapy.

1. Introduction

In the recent years, many nanocarriers such as lipid-based formulations, polymeric micelles and polymeric nanoparticles have been employed as drug carriers for tumor therapy [8]. It has been reported that the polymeric nanoparticles have ability to increase the loading capacity, drug stability and therapeutic activity of the anticancer drugs [33]. Biocompatible polymeric nanoparticles “specifically” have received a great attention as smart delivery systems for chemotherapeutic gents because of their biological properties [10]. These nanoparticles are talented to accumulate in the tumor tissue, causing a disorganized vascular construction, referred to as the enhanced permeability and retention (EPR) effect [38]. As a category of polymeric nanoparticles, chitosan nanoparticles (CSNPs) are attractive polymeric nanoparticles particularly due to its low toxicity, low immunogenicity, biocompatibility and biodegradability [6]. CSNPs have been accepted as a promising drug delivery system, attributing to the positively charged of chitosan (CS) polymer [17]. Indeed, CSNPs have been applied in pharmaceutical applications to target cancer cells like 5-fluorouracil [28]. Moreover, CSNPs have been used in delivery of vaccine, proteins, and genes [3]. Newly, the interest of the CSNPs as antitumor drug carriers has been increased [32]. It was described that the CS has anticancer activity is due to its interfering with the cell metabolism and inhibition of cell growth [3]. The mucoadhesive properties of CS with tumor membrane provide an efficient anticancer drug delivery [46]. It was reported that the CSNPs in nanosize scale (5–200 nm) and positive zeta potential could exhibit the higher antitumor effects than other's nanoparticles [21]. The antitumor activity of CSNPs could be ascribed to its disrupting of the membrane and inducing of apoptosis [27]. Depending on the particle size, CSNPs can target the tumor tissue through the leaky vascular architecture phenomena, known as the enhanced permeability and retention (EPR) effect [32].

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http://dx.doi.org/10.1016/j.jddst.2016.01.004
1773-2247/

Statins represent a class of drugs that is generally used to decrease the blood cholesterol level [31]. However, statins have several effects like lipid-lowering effects, improvement of the endothelial function, antioxidant activity, anti-inflammatory and anticancer activity [7]. Concerning the anticancer activity, statins are the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, which inhibit the rate-limiting step in the cholesterol biosynthesis [42]. It has been reported that cholesterol is an important component of cell membranes as statins has ability to inhibit the biosynthesis of farnesyl and geranylgeranyl pyrophosphate that are essential in a post-translational modifications of G-proteins involved in cellular proliferation [29]. So, statins are considered as chemopreventive drugs in cancer [22]. Nevertheless, these effects depend on the high local statins level [7]. Targeted delivery of statins could increase the site concentrations to improve efficiency and avoid systemic exposure. Pravastatin (PRV) is hydrophilic statins, which can be stably encapsulated in the CSNPs. It has been observed that PRV has an ability to reduce the proliferation of hepatocellular carcinoma (HCC) lines [23]. HCC is the fifth most common malignancy worldwide and the third cause of cancer mortality. It has been recognized that PRV has an effective antitumor activity of liver cancer compared to other statins [16]. Despite several studies stated the antitumor activity of statins, further studies are required to address this issue.

Thus, the aim of this work was to examine the hypothesis that CSNPs of PRV are innovative therapeutic approaches for treatment of HCC. We take the benefits of CSNPs and PRV for tumor localized drug delivery as cancer targeting. In our knowledge, this combination was never described before. Therefore, PRV loaded CSNPs was prepared and characterized in term of particle size, zeta potential, polydispersity index, drug entrapment efficiency, drug loading, scanning electron microscopy (SEM), in vitro release to get the optimized formula. The antitumor activity of the optimum formula was investigated using HepG 2 cells as an in vitro model for the study of human HCC.

2. Materials and methods

2.1. Materials

Pravastatin was purchased from Riyadh Pharma, Riyadh, Saudi Arabia. Low molecular weight chitosan (70 kDa with the degree of deacetylation 75%–85%). Tetrazolium bromide (MTT), HepG2 cell line and dimethyl sulfoxide (DMSO) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Sodium tripolyphosphate (TPP) and glacial acetic acid were acquired from BDH, UK. Sodium hydroxide and all other chemicals were analytical grade, purchased from Sigma, Germany. All other chemicals used were of reagent grade.

2.2. Preparation of chitosan nanoparticles

The CSNPs were prepared by the ionic gelatin method as previously mentioned [12]. The optimization procedure was done as follows: chitosan (5 mg/ml) was dissolved in 0.5% (v/v) acetic acid solution (0.5% v/v) to make up chitosan concentrations at 2, 2.5, 3, 3.5, and 4 (mg/ml). In order to get a clear solution; this mixture was stirred overnight at room temperature using a magnetic stirrer. The pH of the resulting solution was adjusted to 4.5 using 1 M of NaOH solution. TPP solution was obtained by dissolving in pure water to have TPP solutions of 1, 1.25, 1.5, 1.75 and 2 (mg/ml). Various concentrations of PRV (1, 2.5, 5, 7.5 and 10 mg/ml) in TPP solution were used (Table 1). Further, the optimized PRV concentration was used for CSNPs formulations depending on CS/TPP mass ratios. The mass ratios of CS to TPP were, 3:1, 4:1, 5:1, 6:1, and 7:1 (Table 1). To prepare CSNPs, 2 mL of TPP solution were added to 5 mL of CS solution under probe sonication and then continuous stirring for 30 min at room temperature. The obtained CSNP suspension was centrifuged at 30,000 rpm for 30 min. Supernatant was removed and nanoparticles were washed twice with distilled water. Then CSNP were lyophilized using Freeze-drying in the presence of 0.1% mannitol used as a cryoprotectant using (Alpha 1–4 LD Plus, Martin Christ Gefriertrocknugsanlagen GmbH, Osterode am Harz, Germany). The instrument was set at −60 °C with vacuum pressure less than1 mbar for 3 days. Blank CSNP were prepared without PRV. As a control, PRV solution was prepared.

Table 1
The effect of PRV concentrations and CS:TPP mass ratios on the particle size and zeta potential of PRV loaded CSNPs (mean ± S. D., n = 3).

Codes	PRV (mg/ml)	CS:TPP	Particle size (nm)	PDI	Zeta potential (mV)
PRV concentrations					
F1	1.0	5:1	159.5 ± 13.4	0.238 ± 0.0282	30.2 ± 2.52
F2	2.5	5:1	170.3 ± 15.9	0.279 ± 0.0230	30.6 ± 3.17
F3	5.0	5:1	185.9 ± 17.9	0.332 ± 0.031	32.1 ± 3.11
F4	7.5	5:1	247.1 ± 20.4	0.242 ± 0.026	25.2 ± 2.35
F5	10.0	5:1	270.4 ± 23.3	0.323 ± 0.014	25.6 ± 3.45
CS/TPP mass ratios					
F6	5	3:1	129.8 ± 10.5	0.276 ± 0.037	25.1 ± 2.64
F7	5	4:1	165.1 ± 16.9	0.298 ± 0.028	26.4 ± 3.34
F8	5	5:1	175.7 ± 12.8	0.313 ± 0.0374	28.1 ± 3.22
F9	5	6:1	198.4 ± 14.0	0.292 ± 0.008	30.1 ± 4.05
F10	5	7:1	220.1 ± 20.9	0.452 ± 0.048	33.5 ± 2.71

2.3. Particle size, size distribution and zeta potential

The particle size, size distribution (PDI) and zeta potential of the freshly prepared and lyophilized CSNPs were measured at 25 °C by photon correlation spectroscopy using the Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). The lyophilized CSNPs were redispersed on deionized water by using vortexing for 30 s. The nanoparticle suspensions were diluted, and the analysis was performed by dynamic light scattering (DLS) at a scattering angle of 90°. The results were analyzed as an average value of triplicate (n = 3).

2.4. Entrapment efficiency and drug loading

The entrapment of PRV in CSNPs was determined indirectly by ultracentrifugation method. The sample dispersions were centrifuged using Eppendorf tube at 50,000 rpm and 4 °C for 30 min using Optima™ Max-E, Ultra Centrifuge (Beckman Coulter, Pasadena, CA). The amount of PRV entrapped within CSNPs was calculated by difference between the total amount used (PRV_{total}) and the free amount present in the supernatant (PRV_{free}). The non-entrapped PRV amounts in the supernatant were determined by UV spectroscopy at 238 nm. By using a pre-constructed calibration curve made using serial concentrations of PRV (0.002–0.012 mg/ml) in distilled water ($y = 0.0523x-0.0442$; $R^2 = 0.9981$).

Entrapment efficiency (EE%) and drug loading (DL%) were calculated according to the following equations:

$$EE\% = \frac{PRV_{total} - PRV_{free}}{PRV_{toal}} \times 100 \quad (1)$$

$$DL\% = \frac{PRV_{total} - PRV_{free}}{\text{Total weight}} \times 100 \quad (2)$$