International Journal of Biological Macromolecules 62 (2013) 18–24

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Toxicological study of the Primaquine phosphate loaded chitosan nanoparticles in mice

Rampal Rajera, Kalpana Nagpal*, Shailendra Kumar Singh, Dina Nath Mishra

Division of Pharmaceutics, Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar 125001, India

ARTICLE INFO

Article history:
Received 16 July 2013
Accepted 11 August 2013
Available online 22 August 2013

Keywords:
Primaquine
Nanoparticles
Chitosan

ABSTRACT

Primaquine (PQ) shows activity against the late hepatic stages and latent tissue forms of *Plasmodium vivax* and *Plasmodium ovale*. However, liver targeted PQ delivery may be useful to minimize the dose-limiting blood toxicities and side-effects of PQ. The prime objective of this study was the preparation of PQ loaded chitosan nanoparticles (PQ-CS-NPs) in order to enhance drug tolerance and to reduce dose frequency. The morphological analysis of the chitosan NPs displayed particle size in the range 287–686 nm, polydispersity index in the range 0.338–0.430 and zeta potential between 9.21 and 22.80 mV which indicated good stability. PQ-CS-NPs exhibited EE and LC as 64.28 ± 1.85% and 33.18 ± 0.975%, respectively. The *in vitro* drug release (Batch C7) was 97.80 ± 0.65% after 24 h. After intravenous injection of PQ-CS-NPs in mice, the lethal dose of the PQ significantly reduced when compared to that of free PQ solution.

1. Introduction

The drug delivery systems are designed to deliver the active pharmaceutical ingredient in adequate concentration at right place at right time to elicit the desired therapeutic response. To improve the performance of the drugs, the dosage forms of smaller size (microparticles and nanoparticles) have been developed [1]. Nanoparticles (NPs) are solid colloidal particles ranging in size from 10 to 1000 nm in which active constituents may be dissolved, entrapped, encapsulated or attached to the outer surface of the polymer [2,3]. When the drug is encapsulated by polymers in the NPs, the formulation is called nanocapsule. Whereas, if the drug is uniformly distributed throughout the NPs matrix, then it is called nanospheres [4,5]. They are mainly used to target the drug to the specific sites (at the site of action) and to deliver the drug at controlled and sustained rate, which reduce the dosing frequency and systemic toxicity of potent drugs. For the formulation of NPs, various polymers have been utilized for therapeutic benefit as well as to minimize their side effects. Distribution studies demonstrated that NPs are rapidly taken up by the phagocytes of the reticuloendothelial cells and are preferentially distributed in the liver. Many drugs such as primaquine (PQ), metronidazole, sodium ferulate, and 5-fluoro uracil (5-FU), which act on the liver, had been successfully targeted using nanoparticulate drug delivery system approach for the management of diseases such as malaria, amoebiasis, liver fibrosis, hepatic carcinoma etc. [6,7]. In present study the chitosan was preferred as a carrier due to its non-toxic nature and is able to degrade *in vivo* so that it does not accumulate indefinitely in tissues [8]. Bovine serum albumin (BSA) is a suitable carrier for formulation of NPs and is easily adaptable to human body [9]. Colloidal drug delivery system such as microspheres, leptosomes and emulsions are used as intravenously injected carriers for drug delivery to specific organs or targeted sites within human body [10]. In colloidal system, the size of particle is very important in distribution of drug in human body. Generally, the large particles are easily removed by liver and spleen. The stability of small particles is higher than the larger particles in drug delivery devices. Reducing the size of colloidal particle carriers in the range 100–200 nm may enhance the stability of the carriers and may create the chance of escaping from the vascular system *via* cavities in the lining of blood vessel. This may decrease the concentration of the drug in blood [11,12].

Malaria is characterized by cycles of chills, fever, pain, and sweating. More than 200 species of the genus *Plasmodium* have been identified that are parasitic to reptiles, birds, and mammals. There are five identified species of this parasite causing human malaria, namely, *Plasmodium vivax*, *P. falciparum*, *P. ovale*, *P. malariae* and *P. knowlesi*. Primaquine (PQ) is the only drug, which act against the liver schizonts of parasites. However, PQ have been characterized by the dose limiting side effects like hemolytic anemia, methemoglobinemia, leucocytopenia, leucocytosis, gastrointestinal disturbances and abdominal cramps [6,7]. Therefore, it is important to reduce the unnecessarily load of PQ in blood compartment by targeting of drug into the liver tissue, which may increase the therapeutic efficiency and may decrease the toxicity and dose of the drug. Keeping in view of these, the PQ-CS-NPs were aimed to be formulated which may increase the entrapment of NPs

* Corresponding author. Tel.: +91 9416729190; fax: +91 1662276240.
E-mail address: kalpananagpal@gmail.com (K. Nagpal).

0141-8130/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijbiomac.2013.08.019

Table 1
Specification of different batches of PQ-CS-NPs prepared by ionotropic gelation method.

Batch	Drug (mg)	BSA conc. (mg/ml)	Chitosan conc. (mg/ml)
C1	50	0.5	1.0
C2	50	1.0	1.0
C3	50	1.5	1.0
C4	50	0.5	1.25
C5	50	1.0	1.25
C6	50	1.5	1.25
C7	50	0.5	1.50
C8	50	1.0	1.50
C9	50	1.5	1.50

loaded in the reticulo-endothelial cell (parenchymal cells) of the liver.

2. Materials

Primaquine phosphate was obtained as a gift sample from IPCA Laboratories Pvt. Ltd., Kandivili (Mumbai). Chitosan was obtained as a gift sample from Central Institute of Fisheries Technology, Kochi, India. All other chemicals used during this study were of suitable analytical grade and used as received. For *in vivo* study mice were provided by the disease free animal house, LLRUVAS, Hisar, India. The protocol of animal study was duly approved by the Institutional Animal Ethics Committee (CPCSEA No. 0436) Guru Jambheshwar University of Science & Technology, Hisar, India.

3. Methods

3.1. Preparation of PQ-CS-NPs (NPs)

CS NPs were prepared by modified ionotropic gelation method [4,13]. Briefly, required quantities of bovine serum albumin (Table 1) was added to chitosan solution (in 2% acetic acid) with constant stirring and incubated for 30 min at room temperature. Then 20 mL of the solution of the sodium tripolyphosphate (TPP) (0.30 mg/mL) was added drop wise to above solution while stirring using magnetic stirrer. The prepared nanoparticles were separated by the two cycles of centrifugation (13,000 rpm, 20 min, −4 °C temp.) by cooling centrifuge. Then the pellet was re-dispersed in 10 mL of phosphate buffer saline (PBS, pH 7.4) and 2% (w/v) mannitol was added as a cryoprotectant and freeze dried at −48 °C and 28×10^{-3} bar pressure for 24 h (Christ lyophillizer, alpha 2–4 ld plus) and were then characterized.

3.2. Determination of particle size, polydispersity index and zeta potential

For particle size analysis, the NPs suspension was diluted ten times with deionized water to a favorable concentration. The size and size distribution of drug loaded NPs were elucidated by dynamic light scattering/Photon correlation spectroscopy using coulter counter (Delsa^{TM} Nano Common, Beckman Coulter India Pvt Ltd.). The polydispersity index is a dimensionless number and indicates the width of the size distribution of colloidal dispersion and has a value between 0 and 1(0 reflects monodispersity).

3.3. Transmission electron microscopy (TEM)

The morphology, shape and aggregation of nanoparticulate systems were studied by transmission electron microscopy (TEM). For TEM study, a suspension of lyophilized NPs was prepared in double distilled water and few drops of the suspension were deposited onto a carbon-coated copper grid and immobilized for 1–2 min. After immobilization the excess solution was wicked off with filter paper and sodium phosphotungstate solution (0.2%, w/v) was added for negative staining. The grids were washed with double distilled water twice and dried after the few seconds. And then the morphology of NPs was observed using Philips CM-10 transmission electron microscope (TEM) (FEI, USA) using an acceleration voltage of 200 kV.

3.4. Encapsulation efficiency (EE) and loading capacity (LC)

NPs (equivalent to 10 mg of PQ) were dispersed in 10 ml of phosphate buffer saline (PBS pH 7.4) and kept overnight on thermostatic orbital shaker at 37 °C with continuous shaking [14]. Afterwards, the solution was centrifuged to remove the polymer residue and then the total drug concentration was determined spectrophotometrically (UV–visible-NIR Spectrophotometer, Varian cary-5000) at 206 nm ($n = 3$). Drug LC and EE were determined by the following Eqs. (1) and (2) respectively.

$$\text{Drug loading capacity}(\%\text{w/w}) = \frac{\text{Mass of drug in nanoparticles}}{\text{Mass of nanoparticles}} \times 100 \quad (1)$$

$$\text{Encapsulation efficiency}(\%\text{w/w}) = \frac{\text{Mass of drug in nanoparticles}}{\text{Mass of feed drug}} \times 100 \quad (2)$$

3.5. Differential scanning calorimetry (DSC)

The physical state of drug inside the NPs was investigated by DSC. The thermogram of the drug loaded NPs were obtained using DSC (TA instruments, Model no. Q10). For this, the small amount (2–7 mg) of sample was sealed in the aluminum pan and the temperature was raised at 10 °C/min from 40 to 300 °C under nitrogen (20 ml/min) atmosphere.

3.6. Fourier transform infrared spectroscopy (FT-IR)

FT-IR of the different formulations was performed to check the drug-excipient interaction using KBr pellet method (Nicolet 6700 FT-IR Spectroscope).

3.7. X-Ray diffraction studies (XRD)

XRD studies were carried out to study physical changes in the drug during formulation. The powder XRD pattern was traced employing X-ray diffractometer (Table Top XRD Miniflex-2, Rigaku Corporation) for the samples using Cu K (α radiation, a voltage of 30 kV, a current of 15 mA). The samples were analyzed over 2θ range 10°–80° with scanned step size of 0.0170° (2θ) and time 20 s.

3.8. In vitro drug release study

Drug release profile was determined using a modified dialysis technique as shown in Fig. 1 [15]. The setup comprised of a 25 ml glass tube fixed with dialysis membrane (Himedia, dialysis membrane-135) at the bottom, which is immersed in a beaker containing 400 ml 7.4 pH PBS buffer (receiver compartment). Dialysis setup was kept at 37.5 °C temperature and inner tube was rotated at 50 rpm throughout the experiment Drug-loaded NPs (equivalent to 5 mg loaded drug) were dispersed into10 ml of PBS buffer (pH 7.4) and poured into the glass tube (donor compartment). Aliquots (5 ml) were withdrawn at specified time intervals (0, 0.5, 1, 2, 4, 6,