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Protocols

Near-infrared mediated quantum dots and paclitaxel co-loaded nanostructured lipid carriers for cancer theragnostic

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ABSTRACT

Timing is an important factor in cancer management. Theragnostic systems have benefit of improving patients' life-quality by expediting therapeutic decisions. The objective of this study was to explore the potential of co-loaded [quantum dots (CdTe/CdS/ZnS) and paclitaxel] NLC (nanostructured lipid carriers) as a parenteral multifunctional delivery system. The co-loaded NLC was prepared by emulsion-evaporation and low temperature-solidification method utilising glyceryl monostearate, oleic acid, and soya phosphatidylcholine as lipid matrix. In characterising the co-loaded NLC, physicochemical properties of particle size, polydispersity index (PDI), zeta potential (ZP), morphology, encapsulation efficacy (EE) and drug loading (DL) were investigated. Moreover, in-vitro paclitaxel release profile, cytotoxicity, histopathological, in-vivo anti-tumour efficacy, and *in-vivo* and ex-vivo fluorescence optical imaging abilities of the co-loaded NLC were assessed. The mean particle size, PDI and ZP were reported to be 115.93 ± 1.61 nm, 0.17 ± 0.04 and -0.22 ± 0.03 mV, respectively. The particles were spheroid-like in shape with relatively smooth surface. A higher EE ($80.70 \pm 2.11\%$) and DL ($4.68 \pm 0.04\%$) were recorded. The coloaded NLC exhibited a biphasic pattern of drug release. IC_{50} value was found to be 1.05 ± 0.58 μM. The tumour growth inhibition rate of 77.85% was registered. The in-vivo and ex-vivo imaging results indicated capability of the co-loaded NLC to specifically target and detect the H22 tumour. Tissues showed no significant cytoarchitectural differences. We can satisfactorily conclude that co-loaded NLC formulation can be qualified as a splendid parenteral drug delivery system foundation for cancer theragnostic.

1. Introduction

Cancer, a leading cause of death worldwide [1], has devastating health outcomes and economic constraints to humanity [2]. A solid malignant tumour, Hepatocellular carcinoma (HCC) is the third and ninth common cause of cancer related mortality in men and women, respectively [3]. Currently, at the behest of early diagnosis, surgical intervention is the only viable therapy considered to provide cure [4]. However, only 10-20% of HCC can be surgically excised [5]. Therefore, chemotherapy remains an alternative treatment for a larger portion of HCC [6]. Paclitaxel (PTX) is one of the most effective anticancer drugs for the past few decades and has been clinically used in the treatment of various cancers, including HCC [7]. Prognosis of HCC patients is extremely poor [8], with 5-year survival rate of only 5% [9]. Thus, new therapeutic strategies are imperative to combat HCC.

Theragnostic approach may be a better way, since by providing image guided therapy, theragnostics have the ability to monitor effectiveness of therapeutic response, thus expediting therapeutic decisions while improving patients' life-quality [10]. As the name suggests, two agents are required, namely therapeutic and diagnostic agents. For this study, PTX and quantum dots (QDs) were chosen. It is widely acknowledged that PTX is a drug of choice for treatment of various human cancers. The unique physicochemical features of QDs make them extremely attractive fluorophores for *in vivo* imaging of living organism [11]. They have high resistance to photobleaching and high level of brightness [12]. Cadmium-free QDs suffer from poor stability and inferior photophysical properties compared to high-quality QDs made of materials such as CdTe. Although toxicity of CdTe has been reported [13], improving biocompatibility of potentially toxic QDs probes remains a sound

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option [14]. Consequently, passivating agent, ZnS has been used. ZnS shell also importantly enhances colloidal stability of the particles [15].

Unlike other lipid systems like solid lipid nanoparticles, nanostructured lipid carriers (NLC) provide benefits of improved drug loading and controlled drug release [16] while simultaneously ushering a golden platform for designing a multifunctional nanoparticle with potential to merging imaging and therapeutic functionalities within a single unit. The lipid matrix constituted glyceryl monostearate (GMS), soya phosphatidylcholine (SPC) and oleic acid (OA). It was envisaged that the lipid matrix would provide protection from degradation for both PTX and QDs prior to their desired destined sites. More also, as SPC and GMS harbour emulsifying characters, GMS forms a more rigid surfactant film [17]. OA increases mobility and fluidity of the surfactant layer due to its low melting point [18] and thus, promoting formation of small-particles [19]; a feature critical for transport passage. In addition; OA, GMS and SPC have GRAS (generally regarded as safe) status [20].

Herein, we report a novel multifunctional co-loaded (PTX and QDs) NLC (Fig. 1A). The photoluminescent nanocrystals, QDs; CdTe/CdS/ZnS were used. The purpose of this study was to explore the potential of the co-loaded NLC as a parenteral multifunctional delivery system by evaluation of its characteristics *in-vitro*, *in-vivo* and *ex-vivo*. We posit that a stable multifunctional co-loaded NLC potential for use in cancer theragnostic can be achieved.

2. Materials and methods

2.1. Materials

Paclitaxel-PTX (Purity > 99%) was purchased from Shandong Chenxin Pharmaceutical Co., Ltd (Jinan, China). Quantum dots-QDs (CdTe/CdS/ZnS) were purchased from China Beijing Beida Jubang Science & Technology Co., Ltd (Beijing, China). Soya phosphatidylcholine (SPC) was provided by Shanghai Taiwei Pharmaceutical Co., Ltd (Shanghai, China). Glyceryl monostearate (GMS) was purchased from Tianjin Sitong Chemical Company (Tianjin, China) and oleic acid (OA) was purchased from Tianjin Damao Chemical Agents Company (Tianjin, China). Pluronic® F68 (F68) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Non-ionic surfactant polysorbate 80 (Tween-80) was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). HPLC grade acetonitrile was purchased from Cinc. High Purity Solvents Co., Ltd (Shanghai, China). HPLC grade distilled water (Jinan, China) was used throughout the study. All chemicals and solvents were of analytical reagent or HPLC grade.

2.2. Preparation of co-loaded (PTX and QDs) NLC and blank-NLC (b-NLC)

The desired amounts of GMS, OA, SPC, PTX and QDs were accurately weighed, measured and quantitatively transferred into 2 mL eppendorf tube where they were dissolved in 1 mL of acetonitrile. The eppendorf tube was then submerged in a water bath at 80 °C. The resulting organic phase was slowly (8 mL/h) injected by micro-syringe pump (kd Scientific, Holliston, MA, USA) into 10 mL of 0.5% w/v F68 aqueous solution, under mechanical agitation (RCT basic, Guangzhou, China) of 1000 rpm in a water bath at 80 °C for 10 min to form a coarse emulsion. The warm primary coarse emulsion was further treated with a sonicator for 20 min to form a homogenous nanoemulsion. The resulting nanoemulsion was cooled down in an ice (0 °C) bath to produce nanoparticle dispersion [co-loaded (PTX and QDs) NLC] and later stored at 4 °C until use. The b-NLC was the one in which PTX was not added.

2.3. Physicochemical characterisation of the co-loaded NLC

2.3.1. Particle size, polydispersity and zeta potential measurements

The mean particle size, polydispersity and zeta potential of the co-loaded NLC were established by photon correlation spectroscopy using Malvern Zetasizer (3000 HS, Malvern Instruments Ltd., Worcestershire, UK) at 25 °C and 90° scattering angle. The samples were prepared in triplicates, measured and averaged.

2.3.2. Morphology analysis

The morphology of the NLC was observed by transmission electron microscopy-TEM (H-7000, Hitachi, Japan). In brief, a small drop of dilute particle dispersion was spread onto a carbon film coated on a copper grid followed by negative staining with a drop of aqueous solution of 2% sodium phosphotungstic acid for 30 s for contrast enhancement. The samples were dried at room temperature prior to TEM examination.

2.3.3. Determination of encapsulation efficacy (EE) and drug loading (DL)

The desired amount of co-loaded NLC was dispersed in 2.9 mL of 0.5 wt% Tween 80- phosphate buffered saline (pH 7.4) and agitated (XW-80A vortex, Instruments factory of Shanghai Medical University, China) for 3 min to dissolve the free drug. The resulting dispersion was centrifuged at 2500 rpm (3–30 K Sigma, Henderson Biomedical Ltd, London, UK) for 10 min at 4 °C. Upon centrifugation, the amount of the soluble free drug in the supernatant was harvested and measured by HPLC. The HPLC assay (Agilent 1100 series, USA) was performed on a reverse phase C18 analytical column (4.6 mm × 250 mm, pore size 5 μm, InertSustain®, Tokyo, Japan). The mobile phase was a mixture of acetonitrile: water (65:35, v/v) delivered at a flow rate of 1.0 mL/min. PTX was detected at 227 nm with a variable wavelength detector (VWD). The calibration curve for quantification of PTX was linear ($R^2 = 0.9988$) over a range of standard concentrations between 1.0 and 50 μg/mL. The EE and DL were calculated according to the following formulae;

$$EE(\%) = [(W_{total} - W_{free)/}W_{total}] \times 100 \quad (1)$$

$$DL(\%) = [(W_{total} - W_{free)/}W_{lipid}] \times 100 \quad (2)$$

where W_{total}, W_{free}, and W_{lipid} are the weight of drug added in the system, analysed weight of drug in supernatant and weight of lipid added to the system, respectively.

2.4. In vitro *drug release study*

The release profile of PTX from co-loaded NLC was investigated using dialysis bag diffusion technique. In a bid to ensure that impurities like metal ion were removed, dialysis bag was pretreated by thoroughly washing it with 200 mL boiling distilled water containing 2% $NaHCO_3$ and 2 mL of EDTA (1 μM) twice for 10 min each time and soaked overnight prior to the study. Practically, a known amount of PTX loaded nanoparticles (1.429 mL of co-loaded NLC dispersion- 0.35 mg/mL) was placed into the pretreated dialysis bag with 14 kDa molecular cut-off. The bag was incubated with 15 mL of release medium [1 M sodium salicylate in phosphate buffered saline (PBS), pH 7.4] for providing sink condition throughout the release test at 37 °C under horizontal constant shaking at 100 rpm [21]. At predetermined time intervals (0, 1, 2, 4, 8, 24, 48, 72, 96, 120 and 144 h), the dialysis bag was taken out and replaced into a new container filled with 15 mL of the fresh medium to prevent saturation. Of the release sample, 1.0 mL was collected and the concentration of PTX in the medium was analysed by HPLC method as above. A volume of 1.0 mL PTX solution of Taxol® formulation (6 mg PTX/mL in Cremophor EL/ethanol, 1:1, v/v) was diluted with PBS to