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Defining cisplatin incorporation properties in thermosensitive injectable biodegradable hydrogel for sustained delivery and enhanced cytotoxicity

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ABSTRACT

Injectable thermoreversible chitosan (CS)/β-glycerophosphate (β-GP) hydrogels were developed for prolonged localized delivery of cisplatin (Cis). The effects of formulation variables on the thermoreversible hydrogels preparation as well as the impact of drug incorporation method on Cis release were studied. Antitumor activity of Cis CS/β-GP thermoreversible hydrogels were evaluated against HCT-116 human colorectal cancer cells and MCF-7 human breast cancer cells. Incorporation of Cis to CS solution adjusted at pH 6.2 prior to hydrogel preparation deemed necessary to achieve a sustained release up to 4 days. Cis loaded CS/β-GP thermoreversible hydrogels showed enhanced antitumor activity with about 1.2 fold and 2.05 fold that of Cis solution against HCT-116 cancer cells and MCF-7 cancer cells respectively. The obtained enhanced antitumor activity elected this delivery system for further *in vivo* and toxicological investigations.

1. Introduction

Thermoreversible injectable systems have gained attention due to their non-invasiveness, compared to the other localized implantable systems, with the ability to carry therapeutic agents for site specific delivery, prolonged drug action and improved patient compliance (Alexander et al., 2013; Supper et al., 2014). Their ability to deliver chemotherapeutic agents intratumorally or intralesionally has been explored as a potential strategy to maximize anti-tumor effect, reduce systemic toxicity providing a continuous and sustained drug delivery (Kim et al., 2010). Chitosan (CS)/β-glycerophosphate (β-GP) solutions with sol–gel transition temperature close to 37 °C, the physiological body temperature, have found applications in the interstitial delivery of many chemotherapeutic agents (Ruel-Gariepy et al., 2002; Berrada et al., 2005; Kim et al., 2010).

Cisplatin (Cis) is used as first line chemotherapy against various cancers including glioblastomas, metastatic melanomas, peritoneal and pleural mesotheliomas (Boulikas and Vougiouka, 2004). It had also been found to improve the outcome of triple-negative breast cancer therapy (Ozkan et al., 2012). The antitumor properties of Cis are attributed to the kinetics of its chloride ligand displacement reactions (Siddik, 2003; Feng et al., 2007). It interacts with guanine and adenine N7 atoms located in the DNA major groove, leading to DNA bending and interfering with its replication, transcription as well as other nuclear functions, thus, arresting cancer cell proliferation and tumor growth (Cepeda et al., 2007). Despite its clinical success, intravenous Cis administration can lead to nephrotoxicity, bone marrow toxicity, intractable vomiting, peripheral neuropathy, deafness, seizures and blindness (Genc et al., 2014; Wang et al., 2014). Drug resistance during therapy is another important limitation to its use requiring the use of increasing doses (Dzamitika et al., 2006). Locoregional administration of Cis solution via intraperitoneal, transarterial or intratumoral

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administration is not practical as the drug rapidly passes into the blood, thus, limiting its retention time at the tumor site (Konishi et al., 2003).

In this work, CS/β-GP was suggested as a platform delivery system for the chemotherapeutic agent Cis. Gelation of the formulation post injection, providing high sustained local concentration of Cis was thought to increase efficacy, reduce systemic toxicity and offer the possibility of less frequent drug administration. During the study, we focused on the problems associated with Cis incorporation in the injectable CS/β-GP hydrogel. The outcomes arising from the use of two methods of drug incorporation had been characterized and tailored so as to solve obstacles hindering drug inclusion in the selected systems. The adjusted system was tested in different human cancer cell lines.

2. Materials and methods

2.1. Materials

Cisplatin (Cis): QILU Pharmaceutical Co. Ltd China; chitosan (CS) high molecular weight (HMW): MW 310,000 to >375000 daltons, degree of deacetylation (DD) = 78.05%; CS low MW (LMW): MW 50,000–190,000 daltons, DD = 94.2%, β-glycerophosphate (β-GP) disodium salt and glacial acetic acid: Sigma–Aldrich Company, St. Louis USA. Methanol (HPLC grade): Riedel-de Haen Gmbh, Germany. All other chemicals and reagents were of analytical grade.

2.2. Preparation of thermoreversible Cis CS/β-GP hydrogel

Serial concentrations ranging from 0.1 to 1 g/mL of sterilized ice-cold β-GP solutions in deionized water were added dropwise to acidified dialyzed and autoclaved CS solution prepared in 0.75% v/v acetic acid (in a volume ratio of 9:1 of CS: β-GP). The solutions were stirred mechanically at 500 rpm for 15 min in an ice bath and then their gelation temperatures were determined (Berrada et al., 2005; Diao et al., 2011). To study the effect of sodium chloride (NaCl) on the gel, CS was dissolved in a mixture of 0.75% v/v acetic acid and 0.9% w/v NaCl in a volume ratio of 2:1 and the gel was prepared as above.

The drug, used in a concentration of 1% w/v of the gel, was incorporated by either of two methods: Cis was added to CS solution containing NaCl prior to mixing with β-GP solutions. When required, the pH was adjusted using 1 N sodium hydroxide (NaOH), and the solutions were then treated as before and this was labeled as method I. In method II, Cis was added to the ice cold premixed CS/β-GP solutions containing NaCl (Zhou et al., 2008).

2.3. Characterization of Cis CS/β-GP thermoreversible hydrogel

2.3.1. Determination of pH

The pH values of dialyzed CS solution, Cis/CS solutions, plain hydrogels and Cis hydrogels were measured using a pH meter (Genway Ltd., UK). For plain and Cis loaded CS/β-GP systems, measurement was done in an ice bath to ensure sol state.

2.3.2. Gelation temperature and time

A simple test tube inverting method was employed to determine the occurrence of sol-to-gel transition using a thermostatically controlled water bath (Poly science 9006, USA) and the gelation temperature was measured. The gelation time at 37 °C was also recorded (Zhou et al., 2008; Ta et al., 2009).

2.3.3. FT-IR

FT-IR spectra of plain CS/β-GP thermoreversible hydrogels showing gelation temperatures below physiological temperature (37 °C) were obtained at 5 °C (sol-state) and 37 °C (gel-state) using an FT-IR spectrometer (JASCO 4000, USA) in the range 4000–400 cm^{-1}. Spectra of CS/Cis solutions prepared at different pH values ranging from 5.7 to 6.2 with 0.1 increment were also recorded. Samples were placed on NaCl film in a liquid cell assembled holder in the IR laser beam.

2.3.4. Scanning electron microscopy

Following gel formation by incubating the samples in a water bath at 37 °C, selected plain and Cis loaded thermoreversible CS/β-GP hydrogels were frozen and freeze dried for 48 h using a bench top freeze dryer (BenchTop Manifold, Millrock Technology, Inc., USA). The obtained powders were coated with gold under vacuum, and examined by scanning electron microscope (SEM) (JXA-840A, Japan).

2.3.5. In vitro *Cis release study*

A modification of a method previously reported by Bhowmik et al. (2011) was used. Briefly, 1 mL of Cis loaded thermoreversible CS/β-GP solution was placed in a dialysis membrane (cut-off 1000 Da) and allowed to gel in an incubator at 37 °C. The gel was then placed in a stoppered conical flask containing 50 mL phosphate buffer saline (PBS), pH 7.4, incubated in a thermostatically controlled shaker at 50 ± 1 strokes/min. At predetermined time intervals for a period of 14 days, aliquots of 1 mL were withdrawn from the release medium and replaced with the same volume of fresh buffer. The samples were assayed using HPLC (Agilent 1100, Germany) equipped with G 1311 A quaternary pump and UV detector (VWD-G1314 A). A reverse phase C18 column (Thermo® BDS, 250 × 4.6 mm, 5 μ) was used at 25 °C. The wavelength of the UV detector was set at 210 nm and the flow rate of the mobile phase, water:methanol (80:20 v/v), was 1 mL/min. The coefficient of determination (R^2) of the drug calibration curve in PBS in the concentration range of 0.5–200 μg/mL, was 0.998 and the respective limits of detection (LOD) and quantification (LOQ) were 0.3 and 0.5 μg/mL. The CV% ranged from 1.42 to 12.78% and the accuracy for Cis determination was within acceptable range (not more than 6%) with mean% drug recovery of 97.97%.

The release kinetics from the prepared formulae were assessed using Peppas equation (Peppas, 1985):

$$\frac{M_t}{M} = Kt^n \quad (1)$$

where M_t/M is the fraction of drug that has been released at time t, k is a kinetic constant and n is termed the diffusional exponent related to the release mechanism; n equals to 0.5 for diffusional (Fickian) release, 1 for zero-order kinetics and $0.5 < n < 1$ for anomalous (non-Fickian) release.

2.4. Determination of Cis–CS solutions viscosities

The viscosities of Cis–CS solutions prepared at different pH values ranging from 5.7 to 6.4 were measured at 25 °C using a cone and plate viscometer fitted with spindle 52 (Brookfield DV-III ultra-programmable cone and plate rheometer controlled with Brookfield Rheocalc operating software, U.S.A.) at 5 rpm.

2.5. Determination of free cisplatin

Each of the prepared Cis–CS solutions was dialyzed against PBS pH 7.4 using a dialysis membrane (cut-off 1000 Da). After 3 days, free Cis was determined in the dialysate using the validated HPLC method.