International Journal of Pharmaceutics 409 (2011) 185–193

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Doxorubicin loading and eluting characteristics of bioresorbable hydrogel microspheres: In vitro study

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ARTICLE INFO

Article history:
Received 3 November 2010
Received in revised form 23 February 2011
Accepted 26 February 2011
Available online 4 March 2011

Keywords:
Doxorubicin
Ion exchange
Bioresorbable
Hydrogel microspheres

ABSTRACT

Non-bioresorbable drug eluting microspheres are being increasingly used for the treatment of unresectable liver tumors, whereas bioresorbable microspheres have not received much attention. In this study, bioresorbable microspheres prepared from chitosan and carboxymethyl cellulose were loaded with doxorubicin (Doxo) via ion-exchange interactions with carboxylic groups in the microspheres. With a 25–40% decrease in the microsphere size depending on their size ranges, the microspheres could load a maximum of 0.3–0.7 mg Doxo/mg dry spheres. As confirmed by confocal microscopy, Doxo was mainly concentrated in the outer 20 ± 5 μm surface layer of the microspheres. The loaded microspheres were stable in aqueous dispersions without aggregation for a prolonged period of time but degradable in a lysozyme solution. Furthermore, the loaded microspheres exhibited a noticeable pH-sensitive behavior with accelerated release of Doxo in acidic environment due to the protonation of carboxylic groups in the microspheres. Compared to commercial non-resorbable drug eluting beads, the loaded bioresorbable microspheres showed a sustained release manner in phosphate buffered saline (PBS). The release data were fitted to an empirical relationship, which reveals a non-Fickian transport mechanism ($n = 0.55$–0.59). These results demonstrate that the bioresorbable microspheres are promising as attractive carriers for Doxo.

1. Introduction

Hepatocellular carcinoma (HCC), is the most common primary malignant tumor of the liver. It currently represents approximately 6% of all newly diagnosed cases of cancer worldwide (Lewis et al., 2006). Transarterial chemoembolization (TACE) is the most widespread available palliative treatment, which involves the injection of a chemotherapeutic agent followed by an embolic material into the hepatic artery. The embolic agent will occlude the arterial blood supply to the tumor, resulting in an infarct and subsequently necrosis of the tumor (Kettenbach et al., 2008). However, there is no standard procedure for TACE since there are so many variables. Besides, when administered at high dose systematically, most anticancer drugs can cause severe toxicity to the body, including myelosuppression, nausea, mucositis, hair loss, vomiting, and even irreversible cardiac toxicity (Bibby et al., 2005). With the traditional TACE, it is difficult to control the contact time of cancer cells and the chemotherapeutic agents without damaging the hepatic microcirculation. More importantly, it is also hard to predict the release of the chemotherapeutic agent over time. It is reported that drug-eluting beads would enable a more reproducible procedure for TACE of hepatomas (Parkin et al., 2005) due to achievable high concentrations and prolonged drug release in the tumors. Currently, in the United States, the only commercially available drug-eluting bead is the non-bioresorbable DC bead (Biocompatibles, UK), composed of PVA based hydrogel (Lewis et al., 2007). Another nonresorbable drug eluting beads available in Europe and Japan is Quadrasphere™ microsphere (Lee et al., 2010) (BioSphere Medical, Inc., MA). The rationale for bioresorbable drug eluting microspheres is to embolize the artery and release chemotherapeutic agent to the tumor only during its healing process. Meanwhile, the mass and mechanical strength of the microspheres decrease with time, and the component material will be gradually absorbed by the surrounding tissue. Moreover, bioresorbable microspheres would render subsequent access for administration of chemotherapy with respect to the cell cycle (Tsochatzis et al., 2010). Altogether, bioresorbable microspheres enable longer-term delivery of drugs to the surrounding tissue and tumor internal reservoir and abolish the need for a second surgery to remove the device.

There is currently no commercial resorbable drug eluting beads available. Hence, our group has developed a series of bioresorbable microspheres from oxidized carboxymethyl cellulose and carboxymethyl chitosan (OCMC/CCN microspheres) for

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doi:10.1016/j.ijpharm.2011.02.058

transcatheter embolization (Weng et al., 2010). The results have demonstrated that the microspheres are compressible, biodegradable and injectable. Besides, the microspheres can load dye due to the existence of the functional groups on the microsphere matrix. It is also anticipated that the functional groups on the microsphere can bind with therapeutic drugs. Doxorubicin (Doxo), is the most used effective anticancer drug in current oncological chemotherapy. Although it has high antitumor activity for the treatment of a number of solid tumors, such as bladder, gastric and hepatocellular cancers, similar to most anticancer drugs, Doxo exhibits severe side effects when administered at high dose systematically (Guhagarkar et al., 2010; Hruby et al., 2005). Therefore, some efforts have been made to deliver Doxo to targeted tissue by microspheres (Vinchon-Petit et al., 2010; Liu et al., 2001; Tan et al., 2005), micelles (Hruby et al., 2005; Kim et al., 2010; Ye et al., 2008), nanoparticles (Guhagarkar et al., 2010; Qia et al., 2010; Dreis et al., 2007) or synthetic conjugates (Greenwald et al., 2003; Karki and Ostovi, 2004) while minimizing the systemic exposure of the drug. Among various methods, microspheres with charges can load ionic drugs such as Doxo easily by a simple absorption method and show the highest loading for Doxo (Liu et al., 2001). In this work, hydrogel microspheres made of carboxymethyl cellulose and chitosan were used as a vehicle to load and release Doxo. The kinetics of loading and eluting of the microspheres with Doxo have been investigated. The results will provide useful information for the potential application of the bioresorbable CMC/CCN microspheres as a controlled release device for anticancer drugs that can presumably reduce the systematic exposure of Doxo.

2. Materials and methods

2.1. Materials

Chitosan (≥75% deacetylated), sodium carboxymethyl cellulose (M_w = ~700,000), and chicken white lysozyme were purchased from Sigma–Aldrich (St. Louis, MO). Doxorubicin stock solution (2 mg/mL) was obtained from Teva Pharmaceuticals (CA).

2.2. Methods

2.2.1. Preparation of OCMC/CCN microspheres

OCMC was first prepared by our previous method (Weng et al., 2010). In brief, 1 g sodium carboxymethyl cellulose (M_w = ~700,000, Sigma–Aldrich, St. Louis, MO) and 80 mL distilled water were added into a 250 mL flask. After complete dissolution of carboxymethyl cellulose, desired amount of sodium periodate (10%, 25%, and 50% theoretical oxidation degree, as coded OCMC-I, OCMC-II, and OCMC-III, respectively) in distilled water was added into the flask. The reaction was allowed to proceed for 24 h at 25 °C. Then ethylene glycol was added to terminate the reaction. The resulting pre-product was dialyzed with distilled water for 3 days, followed by lyophilizing. The introduction of aldehyde groups was confirmed by treating the product with tert-butyl carbazate followed by ^{1}H NMR (Ito et al., 2007).

CCN was prepared from chitosan (≥75% deacetylated, Sigma–Aldrich, St. Louis, MO) with a method described previously (Chen and Park, 2003). Briefly, into a 3-neck flask, 5 g chitosan, 20 g NaOH, 20 mL distilled water, and 80 mL isopropanol were added. After 24 h, 15 g monochloroacetic acid in 20 mL isopropanol was added. The reaction was allowed to proceed for another 4 h at 50 °C. Then 80 mL ethanol was added into the mixture to stop the reaction. The preproduct was rinsed with 70–90% ethanol to remove salt followed by vacuum drying at room temperature.

OCMC/CCN microspheres were prepared by a modified inverse emulsion-crosslinking method in the presence of a surfactant. Briefly, a CCN aqueous solution of 2 wt% (5 mL) was first mixed with 5 mL OCMC aqueous solution of 2 wt%, which was then added into 50 mL mineral oil containing 1% (v/v) Span 80, to form an emulsion with stirring. The resulting aqueous phase was allowed to evaporate over night at 45 °C with constant stirring. The sediment was isolated by precipitating in isopropanol, followed by centrifugation to remove the oil phase. The resulting microspheres were thoroughly washed with acetone and hexane before vacuum drying. The microspheres prepared from CCN with OCMC in different theoretical degrees of 10%, 25%, and 50% were coded as MS-I, MS-II, and MS-III, respectively.

2.2.2. Swelling of OCMC/CCN microspheres

The swelling of the microspheres was carried out in normal saline. To ensure complete equilibration, dry microspheres (weight = W_{dry}) were allowed to swell for 24 h at 37 °C. Excess liquid drops adhered on the surface were removed by blotting and the swollen microspheres were weighed (W_{wet}). The swelling ratio q was calculated as:

$$q = \frac{W_{wet}}{W_{dry}}. \quad (1)$$

2.2.3. Preparation of Doxo loaded microspheres

The loading of Doxo to the microspheres was performed by immersing the microspheres in a Doxo solution (0.25, 0.5, 0.75, 1, and 2 mg/mL). Briefly, 150 mg swollen microspheres (in normal saline) were added into a 20 mL glass vial, and the excess normal saline was removed as much as possible using a syringe with a 20 G needle before loading. Then 10 mL Doxo solution was added into the vial. The vial was kept at 5 °C in dark during the loading procedure. The change in the drug concentration in the loading solution was monitored by measuring the absorbance at 483 nm at time intervals 5 min, 10 min, 20 min, 30 min, 45 min, 1 day, and 2 day using a UV–visible spectrophotometer (Beckman, DU650) and comparing to a standard curve constructed from Doxo solutions with known concentrations.

2.2.4. Microscopy

The changes in the microsphere size and color during the loading procedure were monitored with a microscope (Nikon) connected to a CCD camera. Fluorescent microscope imaging of the loaded microspheres was conducted with a Zeiss Microscope interfaced with a SPOT camera. Confocal microscopy was performed with an Olympus FluoView FV1000 inverted microscope using Argon 488 nm laser.

2.2.5. Release studies of Doxo from the loaded microspheres

The release of Doxo from the microspheres was conducted in normal saline (pH = 6), acetate buffer (pH = 5.2), PBS (phosphate buffered saline, pH = 7.4), and deionized water, respectively. Briefly, loaded microspheres (3 mg) were added into a disposable plastic cuvette, and then 2 mL releasing medium was added. The concentration of Doxo released in the medium was monitored by measuring the absorbance at 483 nm as described above. The releasing was done at room temperature or 37 °C in dark with and without refreshing the medium. For the experiment with medium replaced, the medium was changed on day 1, day 3, day 6, day 12, day 19, and day 26. The drug releasing was compared to those of the commercial drug eluting beads, DC beads (Biocompatibles, UK).

2.2.6. Enzymatic degradation of the microspheres

The degradation experiment was performed in a lysozyme solution. Briefly, lysozyme was dissolved in 0.01 M PBS solution