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In vitro evaluation of sunitinib loaded bioresorbable microspheres for potential application in arterial chemoembolization

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ABSTRACT

Drug-loadable bioresorbable microspheres (BRMS) are designed for treating hypervascular tumors through chemoembolization, thereby reducing systemic side effects via controllable local delivery. The present study investigated the degradation and loading capability of bioresorbable microspheres with an anti-angiogenic agent, sunitinib, and then evaluated the release profiles in different media (PBS, 10 μg/mL and 4 mg/mL lysozyme solutions), and tested catheter deliverability as well as potential antiangiogenic effects of the loaded microspheres. The dry weight of the BRMS showed a consistent decrease over the period of incubation in a 10 μg/mL lysozyme solution with 61.3% mass remaining on day 21. Sunitinib was loaded efficiently onto the microspheres, with smaller sizes exhibiting a slightly faster loading and release rate. At 2 h, the loading percentages were 99.28%, 97.95%, and 94.39% for 100–300, 300–500, and 500–700 μm microspheres, respectively. At 8 h, the percentage of drug released were 78.4 ± 5.8%, 71.7 ± 0.3%, and 67.0 ± 2.9% for 100–300, 300–500, and 500–700 μm microspheres under static medium conditions, respectively. Under replacing-medium conditions, the presence of 10 μg/mL lysozyme slightly delayed the drug release while 4 mg/mL lysozyme significantly facilitated the drug release from the microspheres as compared with PBS solution. Confocal imaging revealed an even distribution of sunitinib throughout the microspheres. Drug loaded microspheres were delivered through microcatheters smoothly without any clogging. Sunitinib retained its efficacy at reducing the viability of human endothelial cells after elution from the microspheres. Thus, these bioresorbable microspheres are promising for arterial chemoembolization.

1. Introduction

Transcatheter arterial chemoembolization (TACE) has been widely accepted as a minimally invasive treatment for hepatocellular carcinoma [1,2]. Conventional TACE procedure involves the delivery of a doxorubicin in an oil emulsion followed by embolic materials into the tumor vasculature. The choice of embolic agents has been highly variable, including microspherical and nonmicrospherical materials, leading to inconsistent results. In the past decade, the potential benefit of drug-eluting beads (DEBs) over conventional TACE has been discussed in many studies with regard to enhancing the controlled delivery of chemotherapy [3–5]. During this time, attempts at TACE using DEBs allowed for more reproducible results with improved tumor response and reduced drug-related side-effects compared to the conventional TACE [6–9].

In addition to cytotoxic anticancer drugs such as doxorubicin and irinotecan, use of angiogenesis inhibitors has been firmly implemented in the current clinical management of a variety of cancers. For instance, sunitinib is an anti-angiogenic agent approved by the FDA for the treatment of renal cell carcinoma and gastrointestinal stromal tumors [10,11]. Its mode of action is based on the suppression of the tyrosine kinase activity of several growth factor receptors, mainly vascular endothelial growth factor receptor (VEGFR2) and platelet derived growth factor receptor (PDGFR, alpha and beta) [12], which blocks several angiogenesis related pathways [13].

Inhibition of VEGFRs using sunitinib efficiently increased the overall survival in patients with high carbonic anhydrase IX [14]. In a study by Remon et al., oral administration of sunitinib was beneficial in treating patients with advanced thymic malignancies [15].

Sunitinib use, however, is associated with dose-dependent adverse effects, and chronic administration causes problematic side effects, ranging from mild fatigue and hand-foot syndrome

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to life-threatening cardiac toxicity [16,17]. Development of a sustained release formulation of sunitinib using microspheres would be expected to decrease the frequency of administration and lead to improved regimens for the treatment of hypervascular tumors. For these reasons microsphere-based drug delivery systems, which are capable of sustained release, such as DC beads and poly(L-lactide)-*co-polyethylene glycol-co*--poly(D,L-lactide)-(PDLLA-PEG-PDLLA) microspheres, were evaluated for sustained release of sunitinib [13,18]. PDLLA-PEG-PDLLA microspheres are hydrophobic and available only in very small size range between 23 and 34 μm, which could lead to clumping or catheter clogging [13]. DC beads are not resorbable and persist in the body permanently after administration, minimizing or eliminating vessel recanalization and recovery of function in the effected portion of the organ. The permanent blockage by these microspheres also precludes the possibility of repeating TACE procedures, severely limiting follow up treatment options. Additionally the drug release is incomplete, leaving residual sunitinib loaded on the beads long term, which can cause chronic toxicity problems [18]. To overcome these shortcomings, our lab has developed a bioresorbable microsphere (BRMS) from natural polymers, carboxymethyl cellulose and chitosan with additional carboxymethyl groups added [19]. The microspheres are prepared by an inverse emulsion method which results in beads 50 to 1200 μm in diameter. Preliminary results indicate that BRMS are degradable, non-cytotoxic, compressible, colorable, easily injectable, capable of occluding arteries and can be loaded with sunitinib [19–23]. The current study was undertaken to evaluate the drug loading and release properties of BRMS with sunitinib as well as to investigate the potential of BRMS as a platform for anti-angiogenic agent delivery in arterial chemoembolization. This study provides important information supporting the development of a new bioresorbable microsphere for local delivery of sunitinib.

2. Experimental

2.1. Materials

Chitosan (≥75% deacetylated), sodium carboxymethyl cellulose (M_w = ~700,000), and chicken white lysozyme were purchased from Sigma-Aldrich Co. LLC. (St. Louis, MO). Sunitinib base was purchased from LC Laboratories (Woburn, MA).

2.2. Synthesis of modified polymers and microspheres

Carboxymethyl cellulose (CMC) was first oxidized to generate oxidized CMC (OCMC) [20]. In brief, 1 g sodium carboxymethyl cellulose and 80 mL distilled water were added into a 250 mL flask. After complete dissolution of CMC, sodium periodate in 25% molar equivalence in distilled water was added into the flask. The reaction was allowed to proceed for 24 h at 25 °C with protection from light. Then ethylene glycol was added to terminate the reaction. The resulting pre-product was dialyzed against distilled water followed by lyophilization.

Chitosan was then modified to produce carboxymethyl chitosan (CCN) which is water soluble, via a method described previously with slight modification [24]. Briefly, 5 g chitosan, 20 g sodium hydroxide, 20 mL distilled water, and 80 mL isopropanol were placed in a 3-neck flask. After 24 h, 20 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 pre-product was rinsed with 70–90% ethanol to remove salt followed by vacuum drying at room temperature.

BRMS were prepared from OCMC and CCN by a modified inverse emulsion-crosslinking method in the presence of a surfactant [19]. Crosslinking occurred due to a Schiff base formation between the amino groups on CCN and the aldehyde groups on OCMC. Briefly, 5 mL of a 2% (wt) CCN aqueous solution was mixed with 5 mL of a 2% (wt) OCMC aqueous solution and then added into 50 mL mineral oil containing 1% (v/v) Span 80 surfactant to form an emulsion, with stirring at 600 rpm. The resulting aqueous phase was allowed to evaporate over night at 45 °C with constant stirring. The resulting microspheres were sedimented, washed with 5% Tween 80, and isolated by filtration to remove the oil phase, followed by a thorough saline rinse.

Different size ranges (100–300, 300–500, and 500–700 μm) of BRMS were obtained by manual sieving with US standard sieves.

2.3. Enzymatic degradation

Eighteen samples of wet BRMS, 200 mg each, were placed in 22 mL glass vials for a 21 day period of degradation. Ten ml of 10 μg/mL lysozyme in 0.01 M PBS was added to the BRMS and the vials were incubated at 37 °C with mild agitation (50 rpm/min). The degradation medium was changed every other day. At predetermined intervals (day 0, day 3, day 7, day 14, day18, and day 21) three samples were retrieved and rinsed thoroughly with distilled water. The resulting BRMS residues were then lyophilized. The percentage of weight loss was calculated based on the equation: weight remaining (%) = $(W_t/W_0) \times 100\%$, where W_t and W_0 are the dry weight of the BRMS on day t and day 0, respectively.

2.4. Drug loading

The sunitinib loading solution (10 mg/mL) was prepared by first acidifying sunitinib base in a 1.1 molar excess 0.1 N HCl to solubilize the drug in a volumetric flask, and then adding of a 5% (w/v) glucose solution to the meniscus line [18]. Loading of sunitinib onto BRMS was performed by immersing the microspheres in a sunitinib hydrochloride solution. Briefly, 5 g of wet microspheres (hydrated in normal saline) were added into a 20 mL glass vial, and 10 mL of sunitinib solution was added. The vial was kept at ambient temperature in the dark during the loading procedure. The change in the drug concentration in the loading solution was monitored by measuring the absorbance at 430 nm at time intervals 15 min, 30 min, 45 min, 1 h, and 2 h using a UV–visible spectrophotometer (Beckman, DU650) and comparing to a standard curve constructed from sunitinib solutions of known concentrations.

2.5. Drug release

Release experiments were performed in distilled water (DI), saline (0.9% NaCl, pH = 5.6), phosphate buffered saline (0.01 M PBS, pH = 7.4) or lysozyme solutions (10 μg/mL, pH = 7.4; and 4 mg/mL, pH = 7.2) under static medium or replacing-medium conditions. For static medium conditions, BRMS containing 0.1 mg sunitinib were placed in a 4 mL acrylic cuvette, and then 2 mL of medium was added. The cuvette was sealed and kept on a shaker (50 rpm/min) at 37 °C. At different intervals, the absorbance of the liquid in the cuvette was measured with a UV–visible spectrophotometer (Beckman, DU650) at 430 nm. For the replacing-medium condition, BRMS containing 0.5 mg sunitinib were added into a 4 mL acrylic cuvette, and then 2 mL medium (0.01 M PBS, or 10 μg/mL, or 4 mg/mL lysozyme PBS solution) was added. The absorbance of the liquid in the cuvette was measured and the medium was replaced every day.