Biomaterials 61 (2015) 299–306

Contents lists available at ScienceDirect

Biomaterials

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

Poly(lactide-*co*-glycolide) microspheres for MRI-monitored delivery of sorafenib in a rabbit VX2 model

Jeane Chen [a, b], Sarah B. White [b, c], Kathleen R. Harris [b], Weiguo Li [b, d], Jonathan W.T. Yap [f], Dong-Hyun Kim [b, e], Robert J. Lewandowski [b, e], Lonnie D. Shea [a, e, g], Andrew C. Larson [b, e, f, h, *]

[a] Department of Chemical & Biological Engineering, Northwestern University, Evanston, IL 60208, USA
[b] Department of Radiology, Northwestern University, Chicago, IL 60611, USA
[c] Department of Vascular and Interventional Radiology, Medical College of Wisconsin, Milwaukee, WI 53226, USA
[d] Research Resource Center, University of Illinois at Chicago, Chicago, IL 60612, USA
[e] Robert H. Lurie Comprehensive Cancer Center of Northwestern University, Chicago, IL 60611, USA
[f] Department of Biomedical Engineering, Northwestern University, Chicago, IL 60611, USA
[g] Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI 48109, USA
[h] Department of Electrical Engineering and Computer Science, Evanston, IL 60208, USA

ARTICLE INFO

Article history:
Received 25 February 2015
Received in revised form
8 May 2015
Accepted 14 May 2015
Available online 15 May 2015

Keywords:
Sorafenib
Hepatocellular carcinoma
Poly(lactide-co-glycolide)
Magnetic resonance imaging

ABSTRACT

Transcatheter arterial embolization and chemoembolization are standard locoregional therapies for hepatocellular carcinoma (HCC). However, these can result in tumor hypoxia, thus promoting tumor angiogenesis. The anti-angiogenic agent sorafenib is hypothesized to improve outcomes; however, oral administration limits patient tolerance. Therefore, the purpose of this study was to fabricate poly(lactide-*co*-glycolide) microspheres for local sorafenib delivery to tumors during liver-directed embolotherapies. Iron oxide nanoparticles (IONP) were co-encapsulated for magnetic resonance imaging (MRI) of microsphere delivery. Microspheres were fabricated using a double emulsion/solvent evaporation method and characterized for size, sorafenib and IONP content, and MRI properties. MRI was performed before and after intra-arterial microsphere infusions in a rabbit VX2 liver tumor model. The microspheres were 13 microns in diameter with 8.8% and 0.89% (w/w) sorafenib and IONP, respectively. 21% and 28% of the loaded sorafenib and IONP, respectively, released within 72 h. Rabbit VX2 studies demonstrated that sorafenib microspheres normalized VEGFR 2 activity and decreased microvessel density. Quantitative MRI enabled *in vivo* visualization of intra-hepatic microsphere distributions. These methods should avoid systemic toxicities, with MRI permitting follow-up confirmation of microsphere delivery to the targeted liver tumors.

1. Introduction

Hepatocellular carcinoma (HCC) is the most common form of primary liver cancer and globally HCC is the 6th most common cancer [1–3]. Many patients with unresectable HCC undergo transarterial embolization (TAE) or chemoembolization (TACE) procedures wherein a catheter is placed in the femoral artery of the patient and guided selectively to tumor feeding arteries in the liver. Once the catheter is optimally placed, embolic and/or chemotherapeutic agents are co-delivered through the catheter locally to the tumors [4–6]. This allows for specific targeting of tumors and containment of the chemotherapy while also starving the tumor of its blood supply.

Conventional TACE typically involves delivering a mixture of the chemotherapy in Lipiodol® (ethiodized poppyseed oil) with follow-up infusion of embolic particles in an effort to locally contain the chemotherapy by avoiding perfusion-mediated wash-out [4–7]. However, recent studies have shown that after TACE procedures, the creation of ischemic conditions at the tumor promotes a pro-angiogenic response in order to allow the tumor to continue to thrive. This is evidenced by a prominent increase in patient serum VEGF levels within the first 24 h with VEGF levels remaining

* Corresponding author. 737 North Michigan Avenue, Suite 1600, Chicago, IL 60611, USA.
E-mail address: a-larson@northwestern.edu (A.C. Larson).

http://dx.doi.org/10.1016/j.biomaterials.2015.05.010
0142-9612/

elevated for up to one month after TACE procedures [8,9]. This observed phenomenon is correlated to poor patient outcomes. As a result, with the approval of sorafenib (a multikinase inhibitor that targets VEGFR) [10,11] for HCC in 2007, several clinical trials have investigated the efficacy of combining oral systemic administration of sorafenib with TACE in order to address the observed pro-angiogenic response [12,13].

Unfortunately, the systemic distribution of sorafenib is associated with potentially severe side effects such as gastrointestinal symptoms, hand and foot syndrome, and hypertension [14–17]. Clinical studies have indicated adverse events leading to requisite dose reductions in roughly 30% of patients [14,18,19]. Patient tolerance can be severely limited, which in turn limits permitted dose and associated patient response. Local delivery of sorafenib as part of a TACE procedure should be advantageous to locally address the tumor's pro-angiogenic response while improving patient tolerance.

Sorafenib is a hydrophobic drug, thus it is difficult to load sorafenib into pre-existing microsphere platforms such as commercially available hydrogel DC Bead®. In order to formulate sorafenib for TACE procedures, a novel platform is needed. Poly(lactide-*co*-glycolide) (PLG) is a biodegradable polymer already used in FDA approved devices such as Lupron Depot® and in POLYSORB™ sutures. Previous studies have validated the potential to fabricate PLG microspheres for TACE procedures [20–22]. The advantageous drug loading mechanism for PLG platforms involves encapsulating the drug during the fabrication process [23]. PLG microspheres can be loaded with either hydrophobic or hydrophilic drugs, and combined with its biocompatibility [24] can be a platform for translational applications in clinical settings.

The purpose of this study was to develop PLG microspheres that can co-encapsulate sorafenib and iron oxide nanoparticles to enable MRI-monitored local delivery of sorafenib to limit pro-angiogenic responses in liver tumors following transcatheter embolotherapies. After fabrication, the microspheres were characterized for size, loading and release properties before investigating MRI properties *in vitro*. After these initial *in vitro* characterization studies, a rabbit VX2 liver tumor model was employed to enable *in vivo* studies a) validating the potential for MRI-monitored tumor-targeted transcatheter delivery and b) investigating the elicited tumor responses.

2. Materials and methods

2.1. Materials

75:25 Poly (D,L-lactide-*co*-glycolide) (PLG RESOMER® RG 752H, MW = 4000–15000) polymer was purchased from Sigma Aldrich (St. Louis, MO). Sorafenib tosylate was purchased from LC Laboratories (Woburn, MA). The iron oxide was in a ferrofluid solution (EMG 304) and was purchased from Ferrotec (Santa Clara, CA).

2.2. Microsphere fabrication

Microspheres were fabricated via a double emulsion/solvent evaporation method. Specifically, 714 mg of PLG was dissolved in 1.71 mL dichloromethane and added to 71.4 mg of sorafenib in 1.14 mL of DMSO to compose the oil phase. The water phase consisted of a 0.14 mL water-based suspension containing 142 mg of the iron oxide. The oil phase and water phase were combined and homogenized at 7000 rpm for 30 s before adding 15 mL of 1% polyvinyl alcohol. Afterwards, the solution was homogenized again at 7000 rpm for 2 min and poured into a stirred beaker containing 240 mL 0.5% polyvinyl alcohol. The microspheres were stirred in the beaker at least three hours for solvent evaporation before these were collected, washed and lyophilized.

2.3. Microsphere size and morphology characterization

Microspheres were imaged for morphology using a Leica DM IL microscope with a Leica DFC290 Camera (Leica Microsystems, Wetzlar, Germany). Microsphere diameters were determined via Image J software analysis of the resulting images.

2.4. Sorafenib and iron oxide loading

To characterize the amount of sorafenib contained within the microspheres, the microspheres were dissolved in a solution of 60:40 Acetonitrile: 20 mM Ammonium Acetate containing 1% DMSO at a concentration of 1 mg/mL. The samples were then spun down for 5 min at 5000 rpm to separate the sorafenib from the PLG and iron oxide. The supernatant was then analyzed via high performance liquid chromatography (HPLC) using an Agilent 1260 Infinity Quaternary LC HPLC (Santa Clara, CA) system and Zorbax C18 column using the methods as described in Blanchet et al. [25]. HPLC determined sorafenib concentrations were used to estimate the weight percentages of sorafenib in the microspheres by simply dividing the sorafenib concentration by the overall microsphere concentration in the solution analyzed via HPLC. Similar calculations were then used to determine the percentage of sorafenib originally used in fabrication that was actually retained post-fabrication (aka loading efficiency) by dividing the weight percentage by weight percentage of sorafenib used during fabrication.

Similar procedures were performed for characterization of iron oxide content within the microspheres. In triplicate, microspheres were dissolved in nitric acid and then prepared for inductively coupled plasma mass spectrometry (ICP-MS) in 2% nitric acid buffer solutions containing 5 ppb yttrium as an internal standard. Calibration standards were prepared with concentrations of iron ranging from 0 to 100 ppb in 2% nitric acid buffer solution also containing 5 ppb yttrium. ICP-MS was then performed to determine iron concentrations within these microsphere solutions. Similar to sorafenib measurements, weight percentages of iron oxide in the microspheres were determined as the ratio between the mass of iron oxide present in the prepared solutions measured via ICP-MS and the original mass of microspheres dissolved in the nitric acid. This weight percentage was then divided by the original weight of iron oxide used during microsphere fabrication to determine the percentage of iron oxide that was retained within the microspheres post-fabrication (aka loading efficiency).

2.5. Sorafenib and iron oxide release

To study the release kinetics of the microspheres *in vitro*, in triplicate, 5 mg of microspheres were dispersed in 50 mL of 1% sodium dodecyl sulfate in phosphate buffered saline. This solution was then placed in an incubator maintained at 37 °C and at fixed time points 1 mL aliquots were withdrawn and filtered to separate out any microspheres that may have been withdrawn. Any microspheres withdrawn were returned and the 1 mL volume removed was replaced with 1 mL of fresh media to maintain a concentration gradient. The aliquots were then analyzed for sorafenib and iron oxide concentrations via HPLC and the QuantiChrom Iron Assay Kit (BioAssay Systems, Hayward, CA, USA).

2.6. MRI phantom studies

To characterize the T_2^* relaxivity properties of the microspheres, phantoms were created with microspheres embedded in 1% agar gels at concentrations ranging from 0 to 2 mg/mL. These agar gel phantoms were then imaged using a 7 T Bruker Clinscan (Bruker, Billerica, MA, USA) with T_2^* mapping protocol. Specifically a GRE