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Research paper

Promoted antitumor activities of acid-labile electrospun fibers loaded with hydroxycamptothecin via intratumoral implantation

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ABSTRACT

The acidosis of tumor microenvironments is one of the universal phenomena of solid tumors, and the increased acidity may be in fact essential intermediates in the progression of tumor growth and several lethal phenotypic traits of tumors, such as invasion and metastasis. Acid-labile polymers PBELA with incorporating acetal groups into biodegradable backbone of poly(D,L-lactide)–poly(ethylene glycol) (PELA) were utilized to load hydroxycamptothecin (HCPT) into electrospun fibers for intratumoral chemotherapy. Compared with that under a simulated physiological condition of pH 7.4, the incubation of PBELA fibers in acidic media resulted in larger mass loss and molecular weight reduction of fiber matrices and enhanced HCPT release from fibers. *In vitro* cytotoxicity assay of HCPT-loaded PBELA fibers indicated 6-fold higher inhibitory activity against HepG2 cells after incubation in pH 6.8 media than that of pH 7.4, while there was no significant difference for free HCPT and HCPT-loaded PELA fibers. The tumor growth, tumor cell apoptosis, and animal survival rate after intratumoral implantation of HCPT-loaded PBELA fibers indicated a superior *in vivo* antitumor activity and fewer side effects than other treatment. Therefore, acid-labile electrospun fibers may be promising implants for localized therapy of inoperable tumors and for prevention of post-surgical tumor recurrence.

1. Introduction

Chemotherapy is the most curative treatment option for solid tumors along with de-bulking surgery to minimize the risk of recurrence. However, anticancer drugs are usually systematically administered and its circulation within the blood exposes not just tumor cells but all other body organs to the toxicity of the drugs. The drug dose is selected based on the blood volume in the body, and the systemic drug administration usually leads to an inefficient accumulation and penetration into neoplastic cells distant from tumor vessels [1]. So, elevated drug dose must usually be injected in order for the drug concentration in the blood to reach a therapeutic level, which usually results in adverse side effects and compromises the patients' quality of life [2]. To improve the outcome of these cancer patients, a new paradigm of locoregional cancer therapies, including intratumoral infusions, injections, and implantable devices, has rapidly evolved and received considerable attention in the recent years [3]. In addition, for those cancer patients ineligible for surgical resection, drug delivery depot systems for implanting intratumorally or adjacent to the cancerous tissue are considered to be the minimally invasive treatments to release a variety of chemotherapeutic agents for the local therapy [4]. These technologies have been embodied in a variety of formulations such as drug-eluting films, gels, wafers, rods, and particles, featuring predictable and prolonged drug release kinetics [5]. The local regional delivery is designed to maximize destruction to the tumor target by the enriched chemotherapeutic drugs and maintain the therapeutic concentration for a prolonged period of time, while limiting damage to the surrounding normal tissues by reducing the systemic dose [6].

The acidosis of tumor microenvironments, which is induced by glycolysis under hypoxic conditions, is one of the universal phenomena of solid tumors, regardless of tumor types or developmental stages. Compared to normal tissues (pH 7.4), the pH of the tumor extracellular space is in a range of pH 6.0–6.9 [7]. Attempts have been made to develop carriers containing acid-labile linkage, such as acetal, diortho ester and hydrazone linkers, which were formed into micelles, liposomes, nanoparticles, and nanogels [8]. The pH induced destabilization and matrix degradation presents

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a more effective modality of chemotherapy for sensitive tumors by releasing locally high drug concentrations at tumor sites and minimal release during blood circulation (pH 7.4) [9]. Kaminskas et al. conjugated doxorubicin onto polylysine dendrimers through an acid-labile 4-(hydrazinosulfonyl) benzoic acid linker. Less than 10% of the doxorubicin load was released in pH 7.4 buffer over 3 days, while approximately 100% release was evident at pH 5.0. *In vivo* tests on tumor-bearing rats revealed higher uptake into tumors when compared with control tissues such as muscle (around 8-fold) and heart (around 3-fold) [10]. Li et al. synthesized copolymer of poly(N-isopropylacrylamide) and chitosan to load paclitaxel into nanoparticles. Drug release was drastically promoted in tumor surroundings while exerting less effect in normal conditions. For mice treated with nanoparticles, the life span of tumor-bearing mice was significantly increased, and significant tumor regression was observed with complete regression in more than 50% of the mice [11]. Kim et al. encapsulated doxorubicin in mixed micelles composed of poly(L-histidine)-b-poly(ethylene glycol) and poly(L-lactic acid)-b-poly(ethylene glycol)-folate. The pH-sensitivity of poly(L-histidine) segment led to superior efficacy in the cell viability restraint and growth inhibition of multidrug resistant ovarian tumors with minimal weight loss [12].

Electrospun fibers are known as an excellent drug carrier by providing large surface area to volume for high drug loading and encapsulation efficiency, and a three-dimensional open porous structure, which can reduce the constraint to drug diffusion leading to an increase in the total fraction of drug that can be released [13]. In addition, electrospinning affords great flexibility in producing polymer fibers with customizable fiber size, porosity, drug loading and release mechanisms, leading to a possibility to tailor the drug release for each application [14]. Another advantage of drug-loaded electrospun fibers is their potential as implantable device intratumorally, adjacent to tumor, or at the surgical resection margins for cancer chemotherapy of solid tumors. Few attempts have been made in the intratumoral implantation of drug-loaded electrospun fibers to improve the drug availability at the implant surface and to enhance drug penetration into tumors. Ranganath et al. fabricated biodegradable poly(D,L-lactide-co-glycolide) fibrous disks with paclitaxel loaded, and the pharmacokinetics and therapeutic efficacy were evaluated in a subcutaneous [15] and an intracranial model [16]. Enhanced therapeutic paclitaxel penetration was observed in the mouse brain up to 5 mm from the implant site with minimal levels in the plasma, and significant tumor inhibition was determined in comparison with sham and placebo control [16]. In our previous study, hydroxycamptothecin (HCPT) was loaded into electrospun fibers, which were implanted into subcutaneous tumors. Compared with free HCPT, HCPT-loaded fibers provided significantly higher tumor growth inhibition and fewer side effects after intratumoral implantation [17].

Camptothecin is a naturally occurring, pentacyclic quinoline alkaloid that possesses high cytotoxic activities against most of human malignancies such as lung, prostate, breast, colon, stomach, and ovarian carcinomas. Major limitations of the drug, including poor solubility in water and physiologically acceptable organic solvents and hydrolysis under physiological conditions into an open carboxylate form with less antitumor activity and several unpredictable side effects, prevent full clinical utilization [18]. Many attempts have been made to overcome these disadvantages through chemical conjugation, or formulation into liposome, micelle, hydrogel, and nanoparticles [19]. But the loading capability, target accumulation within tumor tissues, and retention of structural integrity are still the major obstacles during the formulation development. In our previous study, HCPT was encapsulated into fibers through blend [20] and emulsion electrospinning [17]. The lactone form of HCPT was maintained around 93% after electrospinning and incubation in buffer solutions due to the core–sheath structure of electrospun fibers [17].

Acid-labile polymers were developed in our previous study by incorporating acetal groups into biodegradable backbone of poly(DL-lactide)–poly(ethylene glycol) (PELA) [21]. Acid-labile nanoparticles with the size of around 200 nm indicated degradation enhancement in response to the acidic environment of endosome, resulting in an efficient escape into cytoplasm for intracellular delivery of quantum dots [22]. Acid-labile microspheres the size of 2–3 μm realized the endosomal escape and released pDNA polyplexes into cytoplasm, promoting transfection efficiency on liver macrophages as antigen presenting cells [23]. In current study, acid-labile electrospun fibers were fabricated to trigger release of HCPT by local acidic microenvironment after intratumoral implantation. The cellular cytotoxicity of HCPT-loaded fibrous mats was assessed after incubation with HepaG2 human hepatocellular liver carcinoma cells at pH 6.8 and 7.4. The *in vivo* antitumor effect was evaluated on hepatic H22 tumor-bearing mice with respect to animal survival, tumor growth, and cell apoptosis.

2. Materials and methods

2.1. Materials

PELA containing 10% of poly(ethylene glycol) (PEG) was prepared by bulk ring-opening polymerization using stannous chloride as initiator [24]. Acetal groups were introduced by reacting PEG with benzaldehyde to obtain poly(benzaldehyde–polyethylene glycol), which was further copolymerized with DL-lactide to obtain poly(benzaldehyde–poly(ethylene glycol))–poly(D,L-lactide) (PBELA) [21]. PBELA and PELA indicated weight-average molecular weight (M_w) of 23.8 and 25.8 kDa, and polydispersity indices (M_w/M_n) of 1.21 and 1.33, respectively. HCPT was purchased from Sichuan Natural Product Co. (Chengdu, China), and 2-hydroxypropyl-β-cyclodextrin (HPCD, M_w: 1396 Da, average degree of substitution: 0.67 hydroxypropyl groups per glucose unit) and bovine serum albumin (BSA) were obtained from Sigma–Aldrich Inc. (St. Louis, MO). Rabbit anti-mouse antibody of caspase-3, goat anti-rabbit IgG–horseradish peroxidase (HRP), and 3,3′-diaminobenzidine (DAB) developer were purchased from Biosynthesis Biotechnology Co., Ltd. (Beijing, China). All other chemicals and solvents were of reagent grade or better and purchased from Changzheng Regents Co. (Chengdu, China) unless otherwise indicated.

2.2. Preparation of HCPT-loaded electrospun fibers

PELA and PBELA fibers containing 3% HCPT and 1% HPCD were prepared by blend electrospinning as described elsewhere [20]. Briefly, HCPT and HPCD were dissolved in dimethylsulfoxide, while PELA or PBELA was dissolved in chloroform. The drug–polymer blend solution was transferred to a 2-mL syringe and then pumped at 1.6 mL/h using a microinject pump (Zhejiang University Medical Instrument Co., Hangzhou, China). A high voltage difference of 20 kV was applied between the syringe nozzle and a grounded collector through a high voltage statitron (Tianjing High Voltage Power Supply Co., Tianjing, China). Fibers were subsequently spun on the aluminum foil wrapped on a rotating mandrel, and the collected fibrous mats were vacuum dried overnight to remove residual solvents and stored at 4 °C, away from light, for further detection.