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Spatial distribution and antitumor activities after intratumoral injection of fragmented fibers with loaded hydroxycamptothecin

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ABSTRACT

There was only a small percentage of drug delivered to tumors after systemic administration, and solid tumors also have many barriers to prevent drug penetration within tumors. In the current study, intratumoral injection of drug-loaded fiber fragments was proposed to overcome these barriers, allowing drug accumulation at the target site to realize the therapeutic efficacy. Fragmented fibers with hydroxycamptothecin (HCPT) loaded were constructed by cryocutting of aligned electrospun fibers, and the fiber lengths of 5 (FF-5), 20 (FF-20), and 50 μm (FF-50) could be easily controlled by adjusting the slice thickness. Fragmented fibers were homogeneously dispersed into 2% sodium alginate solution, and could be smoothly injected through 26G1/2 syringe needles. FF-5, FF-20 and FF-50 fiber fragments indicated similar release profiles except a lower burst release from FF-50. *In vitro* viability tests showed that FF-5 and FF-20 fiber fragments caused higher cytotoxicity and apoptosis rates than FF-50. After intratumoral injection into murine H22 subcutaneous tumors, fragmented fibers with longer lengths indicated a higher accumulation into tumors and a better retention at the injection site, but showed less apparent diffusion within tumor tissues. In addition to the elimination of invasive surgery, HCPT-loaded fiber fragments showed superior *in vivo* antitumor activities and fewer side effects than intratumoral implantation of drug-loaded fiber mats. Compared with FF-5 and FF-50, FF-20 fiber fragments indicated optimal spatial distribution of HCPT within tumors and achieved the most significant effects on the animal survival, tumor growth inhibition and tumor cell apoptosis induction. It is suggested that the intratumoral injection of drug-loaded fiber fragments provided an efficient strategy to improve patient compliance, allow the retention of fragmented fibers and spatial distribution of drugs within tumor tissues to achieve a low systemic toxicity and an optimal therapeutic efficacy.

1. Introduction

Cancer remains a major leading cause of death worldwide although diagnosis and treatment methods have recently been improved to reduce the mortality considerably. After surgery, chemotherapy is the most commonly used treatment strategy for most cancers. However, the conventional systemic cancer chemotherapy suffers from poor pharmacokinetics and inappropriate biodistribution, and only a small percentage of administered drugs reach the target tissues and organs [1]. For example, the low molecular weight of anticancer agents leads to a rapid removal from systemic circulation through renal filtration and a limited accumulation in tumors and tumor cells. In addition, highly hydrophobic drug molecules often have a large volume of distribution, tending to accumulate in and cause toxicities toward many healthy tissues [2]. Thus, cancer chemotherapy usually indicates severe limitations in the safety and effectiveness, such as systemic toxicity, immunogenic injury, and significant morbidity, which have heavy impacts on the quality of life of patients and hamper its wide clinical application. Therefore, many attempts have been made to develop multifunctional carriers to achieve a selective accumulation of chemotherapeutic agents in tumor tissues, cells and subcellular organelles, and subsequently to have an effective anticancer effect with a sufficient therapeutic index [3].

Targeted drug delivery systems have been proposed to overcome biological barriers, intelligently respond to disease environment and release therapeutic agents. One of the targeting mechanisms is passive targeting through drug accumulation into tumors with leaky vasculature and insufficient lymphatic system, referred as the enhanced permeation and retention (EPR) effect

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[4]. This strategy primarily includes long-circulating liposomes, nanoparticles, and micelles, but over 95% of the administered dose is known to accumulate in organs other than tumors, particularly in liver, spleen, and lungs [5]. Alternatively, active drug targeting involves the cell recognition and uptake enhancement by such ligands as folate, transferrin and galactosamine, taking advantage of the overexpression of tumor cell surface receptors. However, the ligand–receptor interactions occur only on the tumor cell surface and do not have the ability to guide themselves to a target, thus the primary mode of tumor localization still relies on EPR-mediated passive extravasation [6]. Previous studies have shown that the presence of tumor-targeting ligands does not always result in an increased accumulation of drug-loaded nanoparticles in tumors [7]. Florence et al. summarizes the current unmet needs and challenges in targeted drug delivery, and raises awareness on the exaggerated claims of the nanoparticle-based drug targeting [8]. Therefore, given that systemic chemotherapy does not have entire ability to guide themselves to a target, it is very difficult to achieve therapeutic levels of drugs within or adjacent to the tumor tissues.

Another strategy to overcome these challenges is known as localized drug delivery, where drug release is limited to the tumor site to maintain therapeutic concentrations of drugs. This makes therapies more efficient with minimal side effects for patients, capabilities of which cannot be achieved by conventional systemic administration of drugs [9]. Over the past decades, implantable drug-releasing systems have become more sophisticated and implants based on silicone rubber or polymers have extensively been used for the administration of steroid hormones, anesthetic agents, antibiotics, anticancer drugs, and insulin [10]. Drug-loaded wafer [11], film composites [12] and fibrous mats [13] have been implanted directly into a tumor or at the site of tumor resection. But the physical implants require direct accessibility through surgical procedures, having a large invasiveness. Alternatively, local injection of nanoparticles, microparticles and *in situ* forming precipitates into tumors or along the perimeter of tumors was developed for cancer treatment. Benny et al. injected microspheres with entrapped antiangiogenic agents into intracranial glioma tumors in nude mice, causing a remarkable reduction in the tumor volume with a significant decrease in angiogenesis and an increase in apoptosis [14]. Zhao et al. evaluated paclitaxel-loaded nanoparticles on A-549 tumor-bearing mice via intratumoral injection, showing an effective inhibition on the tumor growth and a higher cytotoxicity than commercial paclitaxel formulation Taxol [15]. But the injection of microsphere or nanoparticle suspensions indicates a quick migration from the administration site [16]. Another injectable implant is syringeable liquid formulations, which are injected intramuscularly or subcutaneously and solidified *in situ* to form solid or semi-solid drug depots. One commercialized product was based on poly(lactide-co-glycolide) (PLGA) solutions in water-miscible organic solvents containing leuprolide acetate, which were subcutaneously injected for the treatment of prostate cancer. The diffusion of organic solvent toward the surrounding aqueous environment led to PLGA precipitation and formation of an implant system for a sustained release of leuprolide acetate over 6 months [17]. As carrier vehicles, several water-miscible organic solvents, such as N-methyl-2-pyrrolidone (NMP) and dimethyl sulfoxide (DMSO) are the most preferred, but their use is restricted owing to controversial reports regarding the toxicity [18]. Alternatively, DuVall et al. evaluated the thermogelling of polylactide–poly(ethylene glycol) copolymers (PELA) at physiological temperatures to sustain the release of paclitaxel after intratumoral injection. Phase II clinical trials have revealed a successful tumor growth inhibition, good tolerability and low systemic exposure [19]. However, the injectability and gelation process should be balanced in the *in situ* forming hydrogels in response to changes in environmental temperature and pH [20]. It should be noted that the major limitation of these injectable implants was the extensive burst release rightly after injection into tumors, due to the lag time between the injection of the system and the precipitation/gelation of the polymer. Sometimes the drug levels were higher than the recommended safety margin, which is obviously more problematic for those drugs that have a narrow therapeutic window [21].

In this view, fragmented fibers with hydroxycamptothecin (HCPT) loaded were proposed in the current study for cancer treatment after intratumoral injection. Fragmented fibers not only retain the advantages of continuous fibers such as large specific surface areas, localized and controlled delivery, but also have the ability to reduce the invasion of surgical implantation of fiber mats into tumors. Fragmented fibers were constructed by cryocutting of aligned electrospun fibers, and the lengths could be conveniently controlled by adjusting the slice thickness to achieve a good injectability and tissue remaining after local injection. The *in vivo* distribution of HCPT released from fiber fragments was investigated in tumors, blood and other tissues, compared with free HCPT and fiber mats. The spatial distributions of HCPT and fiber fragments of different lengths in tumor tissues were also determined from serial tissue sections. The antitumor efficacy was evaluated on H22-tumor bearing mice with respect to tumor growth inhibition, animal survival, histopathological and immunohistochemical (IHC) analysis of tumors retrieved.

2. Materials and methods

2.1. Materials

PELA (M_w = 50 kDa, M_w/M_n = 1.23) containing 10% of poly(ethylene glycol) (PEG) was prepared by bulk ring-opening polymerization of lactide/PEG using stannous chloride as the initiator [22]. HCPT with purity of over 98% was from Junjie Biomedical Ltd. (Shanghai, China), and collagenase IV, trypsin, and DMSO were obtained from Sigma–Aldrich (St. Louis, MO). Rabbit anti-mouse antibodies of caspase-3 and Ki-67, goat anti-rabbit IgG-horseradish peroxidase (HRP), and 3,30-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 fiber fragments

HCPT-loaded fiber fragments were prepared from aligned electrospun fibers (EF) by cryocutting, and aligned fibers were obtained after collecting on a rotating mandrel as described elsewhere [23]. Briefly, 10 mg HCPT was dissolved in 80 μl of DMSO, while 500 mg of PELA was dissolved in 3.0 ml of chloroform/dimethyl formamide (5/1, v/v). The blend of above solutions was transferred to a 5-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/15 cm 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 deposited on an aluminum foil wrapped on a grounded rotating mandrel at a linear rate of around 15 m/s. After vacuum drying overnight to remove residual solvents, the fibrous mat was folded at about 1 cm of intervals perpendicularly to fiber alignment and was completely soaked with distilled water. The folded fibrous mat was placed vertically in a plastic embedding cryomold, followed by the addition of Cryo-OCT compound (Thermo Fisher Scientific Inc., Waltham, MA) and freezing at −70 °C for 5 min. The solidified block of gels with fibrous mats