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Antimetastasis and antitumor efficacy promoted by sequential release of vascular disrupting and chemotherapeutic agents from electrospun fibers

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ABSTRACT

The vasculature in tumor microenvironment plays important roles in the tumor growth and metastasis, and the combination of vascular disrupting agents with chemotherapeutic drugs should be effective in inhibiting tumor progression. But the dosing schedules are essential to achieve a balance between vascular collapse and intratumoral uptake of chemotherapeutic agents. In the current study, emulsion and blend electrospinning were used to create compartmental fibers accommodating both combretastatin A-4 (CA4) and hydroxycamptothecin (HCPT). The release durations of CA4 and HCPT were modulated through the structure of fibers for dual drug loadings and the inoculation of 2-hydroxypropyl-β-cyclodextrin in fiber matrices. Under a noncontact cell coculture in Transwell, the sequential release of CA4 and HCPT indicated a sequential killing of endothelial and tumor cells. In an orthotopic breast tumor model, all the CA4/HCPT-loaded fibers showed superior antitumor efficacy and higher survival rate than fibers with loaded individual drug. Compared with fibrous mats with infiltrated free CA4 and fibers with extended release of CA4 for over 30 days, fibers with sustained release of CA4 for 3–7 days from CA4/HCPT-loaded fibers resulted in the most significant antitumor efficacy, tumor vasculature destruction, and the least tumor metastasis to lungs. A judicious selection of CA4 release durations in the combination therapy should be essential to enhance the tumor suppression efficacy and antimetastasis activity.

1. Introduction

The aggressive growth of cancer cells and associated overexpression of proangiogenic factors lead to the development of blood vessel networks in tumors. The vasculature in tumor microenvironment not only provides necessary nutrition and oxygen for tumor cell growth and progression, but also plays important roles in the invasion and extravasation of primary tumor cells and eventual metastasis. Therefore, the tumor vasculature is an attractive and potentially valuable target for anticancer therapy by the use of antiangiogenesis and vascular disrupting agents (VDAs) (Bergers and Benjamin, 2003). In contrast to normal vessels, tumor-related vasculature is characterized by a discontinuous luminal layer, high vascular permeability and interstitial fluid pressure. The profound differences make the tumor vasculature more susceptible than normal vessels to the effects of VDAs, leading to an acute and pronounced shutdown of blood vessels, ultimately causing selective tumor necrosis (McKeage and Baguley, 2010).

Combretastatin A-4 (CA4), isolated from the bark of south African trees *Combretum caffrum*, is one of the most clinically advanced VDAs. As a tubulin-binding VDA, CA4 indicates notable effects on the vascular shutdown, and inhibits the tumor growth and metastasis in a wide variety of preclinical tumor models (Zhu et al., 2010). Phase I trials have shown that CA4 can be reasonably tolerated in cancer patients and produce a significant reduction of tumor blood flow (Stevenson et al., 2003). A phase II trial showed that about 25% of thyroid cancer patients experienced more than 3 months of progression-free survival after CA4 treatment (Cooney et al., 2006). However, monotherapy with VDAs cannot control tumor growth and metastasis, due to that peripheral tumor cells can derive sufficient quantities of oxygen and nutrients from nearby normal tissues, and the VDA exposure usually causes cardiovascular adverse events (Busk et al., 2011). Therefore, the combination of VDAs with other chemotherapeutic agents has

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been proposed to inhibit different pathways of tumor progression for either additive or synergistic effects. Zhang et al. (2011) indicated that the combination of tumor necrosis factors and CA4 exerted synergistic antiproliferative effects against human colon carcinoma cells *in vitro* and a xenograft model in nude mice, accompanied by caspase-mediated apoptosis. Yeung et al. (2007) demonstrated strong antineoplastic activities of the combinations of CA4, paclitaxel and carboplatin against anaplastic thyroid cancer, which were consistent with vascular disruption but not direct inhibition of cell proliferation as the primary antineoplastic mechanism contributed by CA4.

There are some obstacles difficult to overcome when combining CA4 and other chemotherapies for cancer treatment. One of the challenges is to achieve a sustained and target release of CA4. Although tumor endothelium is more vulnerable to VDAs, clinical data revealed that normal vascular endothelia seemed to be affected as well, leading to cardiac ischaemia and arrhythmias as well as neurologic complications (Siemann, 2011). Hill et al. (2002) indicated that CA4 had significant effects on the growth of both transplanted and spontaneous murine tumor models after intraperitoneal injection following a frequent low-dose schedule, whereas a single large dose had no measurable effect. The combination of VDAs with chemotherapeutic agents also requires multiple administrations at different schedules to maximize the antitumour activity (Dorrell et al., 2007). Sengupta et al. (2005) conjugated doxorubicin onto poly(lactic-co-glycolic acid) nanoparticles, which were entrapped in pegylated-phospholipid vessel containing CA4. A rapid release of CA4 from the vessel caused a vascular shutdown, followed by a sustained release of doxorubicin from inner nanoparticles resulting in an improved therapeutic index and a reduced toxicity. Similarly, CA4 was physically entrapped in capsules of paclitaxel-conjugated amphiphilic polyester, micelles of doxorubicin-conjugated polylactide copolymers and nanoparticles of methotrexate-conjugated pullulan derivatives (Wang and Ho, 2010; Wang et al., 2011b). The concurrent release of CA4 and chemotherapeutic agents demonstrated promising effects on the disruption of tumor vasculature and inhibition of tumor cell proliferation.

Another challenge is the optimization of doses and schedules in the combination therapy of antivascular and cytotoxic strategies. Generally antivascular therapies aim to reduce tumor blood supply for starvation of tumor cells, while cytotoxic therapies rely on ample blood supply for the access of chemotherapeutic drugs within tumor cells. The vascular collapse may interfere with the drug delivery capability, thereby decreasing the exposure of tumor cells to cytotoxic agents (Dorrell et al., 2007). Additionally, the tumor vascular shutdown may also drive the overexpression of hypoxia-inducible factor-1, which is directly related to the increase in tumor invasiveness and resistance to chemotherapy (Hasani and Leighl, 2011). Therefore, suboptimal dose scheduling may lead to the antivascular and cytotoxic therapies acting against each other in terms of vascular collapse and intratumoral uptake of chemotherapeutic agents. Recent studies have proposed the normalization of tumor vasculature and microenvironment in antiangiogenic treatments to enhance the efficacy of cytotoxic therapies (Goel et al., 2011). But up to now few studies have realized different release patterns of chemotherapeutic and vascular disrupting agents and clarified the effects on the antitumor efficacy and tumor metastasis.

Electrospun fibrous mats with high surface area as well as three-dimensional open porous structure help to reduce the constraint towards drug diffusion, resulting in an efficient drug release system. Another advantage is that drug-loaded fibers can be implanted intratumorally or adjacent to tumor tissues for those unresectable or inoperable solid tumors, or at the resection margins after surgical removal of solid tumors. The local drug release from fibers can enhance the drug accumulation within tumors and decrease the systemic exposure, resulting in a higher therapeutic efficacy and less side effect (Greiner and Wendorff, 2007). Few attempts have been made to entrap dual drugs in fibers and modulate a sustained and programmable release. Coaxial electrospinning of drug-loaded colloids and nanoparticles in fibers, gradual deposition of multilayered electrospun mats and sequential coating of multiple layers on fibers have been proposed to achieve a sequential release of multiple drugs (Jo et al., 2009; Okuda et al., 2010; Kontogiannopoulos et al., 2011). But an uniform coating and nanoparticle distribution in electrospun fibers needed further improvement for an efficient control of drug release (Wang et al., 2011a). Thus, it is indeed challenging in the release modulation of multiple drugs from electrospun fibers.

In our previous studies, hydroxycamptothecin (HCPT) were entrapped with electrospun fibers, indicating higher *in vivo* antitumor activities and fewer side effects after intratumoral implantation than free HCPT injection. Different release profiles of HCPT were demonstrated for blend and emulsion electrospun fibers due to presence of drugs in different locations in fibers (Luo et al., 2012a,b). In the current study, CA4 and HCPT were loaded in the sheath and core regions of fibers, respectively, through a one-step, single-nozzle electrospinning technique. The addition of 2-hydroxypropyl-β-cyclodextrin (HPCD) in the core or sheath regions or both of them in fibers was used to modulate the release of CA4 and HCPT in different profiles. To achieve a harmonious antivascular and cytotoxic therapy, the CA4 release durations were optimized in the combination therapy with respect to the antitumor efficacy and metastasis of tumor cells to lungs in an orthotopic breast tumor model.

2. Materials and methods

2.1. Materials

Copolymer poly(ethylene glycol)-polylactide (PELA, Mw = 42.3 kDa, Mw/Mn = 1.23) containing 10% of poly(ethylene glycol) (PEG) was prepared by bulk ring-opening polymerization using stannous chloride as initiator (Li et al., 2000). CA4 with purity of over 98% was obtained from Great Forest Biomedical Ltd. (Hangzhou, China), and HCPT with purity of over 98% was from Junjie Biomedical Ltd. (Shanghai, China). Collagenase IV, HPCD and bovine serum albumin (BSA) were procured from Sigma–Aldrich Inc. (St. Louis, MO). Rabbit antimouse antibodies of caspase-3 and Ki-67, goat antirabbit IgG–horseradish peroxidase (HRP) and 3,3-diaminobenzidine (DAB) developer were purchased from Biosynthesis Biotechnology Co., Ltd. (Beijing, China), and rabbit antimouse antibody of CD31 was from Abcam (Cambridge, UK). Human umbilical vein ECs and 4T1 were from American Type Culture Collection (Rockville, MD) and maintained in RPMI 1640 medium (Invitrogen, Grand Island, NY) supplemented with 10% heat inactivated fetal bovine serum (FBS, Gibco BRL, Grand Island, NY). All other chemicals and solvents were of reagent grade or better, and received from Changzheng Regents Co. (Chengdu, China), unless otherwise indicated.

2.2. Preparation of electrospun fibers loaded with CA4 and HCPT

Electrospun fibers loaded with both CA4 and HCPT were prepared by blend and emulsion electrospinning as previously described (Luo et al., 2012a,b). The theoretic loading amount was 5% with the CA4 and HCPT mass ratio of 5/1. Table 1 summarizes the composition for each preparation of emulsions and blend suspensions for electrospinning into Fch-1, Fch-2, Fch-3 and Fch-4 fibers. Briefly, PELA, CA4 and HPCD were dissolved in dichloromethane to form an oil phase, while HCPT solution in