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Nanoparticles responsive to the inflammatory microenvironment for targeted treatment of arterial restenosis

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ABSTRACT

Coronary arterial disease (CAD) remains the leading cause of death globally. Percutaneous coronary interventions are frequently used nonsurgical techniques for treating CAD, which may unfortunately lead to arterial restenosis. Currently, there are no effective drugs that can thoroughly prevent restenosis. We hypothesize inflammation-triggerable nanomedicines may function as effective therapeutics for targeted therapy of restenosis, by preferentially releasing their payload at the diseased site. To demonstrate our hypothesis and develop targeted nanotherapies for restenosis, this study was designed to examine effectiveness of nanomedicines responsive to the inflammatory microenvironment with mild acidity and high reactive oxygen species (ROS). To this end, an acetalated β-cyclodextrin (β-CD) material (Ac-bCD) was synthesized as a pH-responsive carrier material, while a ROS-responsive material (Ox-bCD) was produced by hydrophobic functionalization of β-CD with an oxidation-labile group. Based on these two responsive materials, either pH- or ROS-responsive nanoparticles (NPs) were produced by a nano-precipitation technique and fully characterized. Using rapamycin (RAP) as a candidate drug, responsive nanotherapies were fabricated. *In vitro* hydrolysis and release studies confirmed these nanovehicles and nanotherapies exhibited desirable responsive behaviors. Both *in vitro* cell culture and *in vivo* evaluations revealed their good safety profile. These responsive NPs could be effectively internalized by rat vascular smooth muscle cells, which in turn notably potentiated anti-proliferation and anti-migration activities of RAP. After intravenous (*i.v.*) injection, NPs may be accumulated at the injured site in the carotid artery of rats subjected to balloon angioplasty injury. Compared with a non-responsive nanotherapy based on poly(lactide-co-glycolide), treatment with either pH- or ROS-responsive nanotherapy by *i.v.* injection more effectively attenuated neointimal hyperplasia in a rat model of arterial restenosis. Accordingly, nanotherapeutics responsive to the inflammatory microenvironment hold great potential for the management of vascular restenosis by selectively releasing drug molecules at the inflamed sites.

1. Introduction

Coronary arterial disease (CAD) remains the leading cause of death globally, resulting in 8.14 million deaths in 2013 [1]. Percutaneous coronary interventions (PCI) including balloon dilation, excisional atherectomy, and endoluminal stenting are frequently used nonsurgical techniques for treating CAD such as acute myocardial infarction and acute coronary syndrome [2,3]. Unfortunately, regardless of revascularization procedures, thrombosis and/or neointimal hyperplasia generally occurs after initial successful angioplasty, which in turn leads to restenosis, an arterial reobstruction. It has been found that restenosis is responsible for the 30–40% long-term failure rate after coronary revascularization [4]. Inflammation plays a critical role in vascular response to the arterial injury during PCI [5]. Immediately after PCI, endothelial denudation and platelet deposition occur at the injury site [6]. In addition to thrombus formation, activated platelets recruit circulating leukocytes via platelet receptors [7]. Subsequently, the

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recruited leukocytes roll along the injured surface and release several proinflammatory cytokines such as tumor necrosis factor-α and interleukin-6. This in conjunction with factors released from platelets and endothelial cells, such as platelet-derived growth factor (PDGF) and basic fibroblast growth factor, induce migration and proliferation of vascular smooth muscle cells (VSMCs), sequentially enhance extracellular matrix synthesis, and ultimately result in neointima formation. Based on these pathophysiological features, various therapeutics including anticoagulant, anti-inflammatory, and anti-proliferative agents have been investigated to treat restenosis [8,9]. However, for these drugs delivered by systemic administration, only limited efficacy was observed in clinical trials, despite their success in animal studies [10]. This undesirable therapeutic effect has been considered to be mainly caused by rapid drug clearance from the vessel, poor drug accumulation at the site of arterial injury, and inefficiency in maintaining therapeutics in adequate levels for appropriate periods of time as well as relatively low doses administered due to systemic toxicity. Consequently, new delivery strategies and novel therapeutics are necessary to circumvent these drawbacks.

Recently, nanomedicinal approaches have been demonstrated promising for diagnosis and therapy of inflammatory and cardiovascular diseases [11–17]. Nanoparticles (NPs) with different biophysicochemical properties were found effective for the prevention and treatment of restenosis [18,19], by targeting pathological sites of interest. Besides intraluminal delivery of nanomedicines [10,20], different physical and biochemical strategies have been explored for site-specific delivery of therapeutics to the target site by systemic administration [21,22]. To target injured vasculature, different molecular moieties are frequently employed for surface functionalization of NPs, such as collagen IV-targeting peptides [19,23], peptides specific to vascular cell adhesion molecule-1 [24], antibodies to intercellular adhesion molecule 1 [25], ligands that can preferentially bind to activated platelets [26], or their combinations [27].

Whereas significant progress in this field has unambiguously demonstrated the advantages of nanotherapeutics for targeted therapy of arterial restenosis, considerable challenges remain with respect to targeting efficiency, controlled drug release at the diseased site, favorable risk-benefit ratio, and desirable cost-benefit balance from the viewpoint of translation of currently available targeting nanosystems [28–31]. As well documented, overproduced reactive oxygen species (ROS) play a significant role in the pathogenesis of restenosis [32]. Substantially increased ROS levels were found in coronary arteries following balloon injury, which was positively correlated with the development of restenosis [33]. Additionally, mildly acidic microenvironments have been observed at inflamed sites [34]. On the other hand, our previous studies demonstrated that chemical modification of cyclodextrins and their polymers with sensitive groups is a facile and efficient approach to synthesize functional carrier materials [35–38]. For example, materials responsive to mildly acidic pH can be produced by kinetically controlled acetalation of cyclodextrins [38,39], while chemical conjugation of oxidation-labile moieties onto cyclodextins may afford ROS-sensitive materials [36]. Nanoplatforms based on these responsive materials can be employed for encapsulation and triggerable release of a variety of therapeutics varying from small molecule drugs, peptides, to nucleic acids [40–43]. Moreover, *in vivo* evaluations in various animal models indicated that nanovehicles based on these responsive cyclodextrin materials exhibit good safety profile for administration by various routes. Based on these issues, herein we hypothesize nanomedicines that are triggerable by inflammatory microenvironments and can preferentially release their cargo molecules at the diseased site, may function as effective therapeutics for targeted treatment of restenosis (Fig. 1A). To test our assumption, either pH- or ROS-responsive nanotherapies were developed. Both *in vitro* evaluations and *in vivo* studies were performed to interrogate therapeutic benefits of responsive nanomedicines, with rapamycin as a candidate drug.

2. Materials and methods

2.1. Materials

β-Cyclodextrin (β-CD) and lecithin (from soybean) were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). 2-Ethoxypropene (EP) was supplied by Shanghai Beihe Chemicals Co., Ltd. (Shanghai, China). Rapamycin (RAP) was obtained from Beijing Huamaike Biotechnology Co., Ltd (Beijing, China). Pyridinium *p*-toluene sulfonate (PTS), 4-(hydroxymethyl) phenylboronic acid pinacol ester (PBAP), 4-dimethylaminopyridine (DMAP), and 1, 1'-carbonyldiimidazole (CDI) were purchased from Acro Organics. Poly(lactide-co-glycolide) (PLGA, 50:50) with an intrinsic viscosity of 0.50–0.65 was purchased from Polysciences, Inc. (U.S.A.). 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG) was purchased from Corden Pharma (Switzerland). Penicillin, streptomycin, and fetal bovine serum (FBS) were provided by Gibco (Waltham, MA, U.S.A.). Dulbecco's modified eagle medium (DMEM) was obtained from Gibco (U.S.A.). Platelet-derived growth factor-BB (PDGF-BB) was purchased from R&D Systems (U.S.A). Cy5 and Cy7.5 NHS ester were purchased from Lumiprobe, LLC. (U.S.A.). 4,6-Diamidino-2-phenylindole (DAPI) and LysoTracker Green® were supplied by Invitrogen (U.S.A.).

2.2. Synthesis of a pH-responsive material of acetalated β-CD

Acetalated β-CD (Ac-bCD) was synthesized by acetalation of β-CD in the presence of excess amount of EP, using PTS as a catalyst (Scheme S1A) [38,39]. In brief, 4.5 mL of EP (40 mmol) and 160 mg PTS were added into 8 mL of anhydrous DMSO containing 1 g β-CD (0.88 mmol). Acetalation was conducted at 25 °C under magnetic stirring. After 3 h, the reaction was terminated by triethylamine. The product was collected by precipitation from deionized water and centrifugation at 14000 rpm. After thorough rinsing with deionized water, the sample was lyophilized to give a white powder. The obtained material was characterized by ^{1}H Nuclear Magnetic Resonance (NMR) spectroscopy (Agilent 600 MHz) and Fourier transform infrared (FT-IR) spectroscopy (S100, PerkinElmer).

2.3. Synthesis of a ROS-responsive material from β-CD

A ROS responsive β-CD material Ox-bCD was synthesized according to our previously reported method (Scheme S1B) [36]. Specifically, PBAP (2.0 g, 8.5 mmol) was dissolved in dry dichloromethane (DCM) (12 mL), into which 2.8 g CDI (17.0 mmol) was added. After 30 min of reaction, 14 mL of DCM was added into the mixture, and then washed with 10 mL of deionized water three times. The organic phase was further washed with saturated sodium chloride solution, dried over sodium sulphate, and concentrated to obtain CDI-activated PBAP. Ox-bCD was then synthesized by conjugating PBAP units onto β-CD. To this end, 250 mg β-CD (0.22 mmol) was dissolved in 20 mL of anhydrous DMSO, into which 0.8 g DMAP (6.6 mmol) was added, followed by addition of 1.5 g CDI-activated PBAP (4.6 mmol). Thus obtained mixture was magnetically stirred at room temperature for 12 h. The final product was obtained by precipitation from water, and collected by centrifugation. After thorough rinsing with deionized water, the sample was lyophilized to give a white powder. Similarly, the final