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Non-proinflammatory and responsive nanoplatforms for targeted treatment of atherosclerosis

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ABSTRACT

Atherosclerosis is the leading cause of many fatal cardiovascular and cerebrovascular diseases. Whereas nanomedicines are promising for targeted therapy of atherosclerosis, great challenges remain in development of effective, safe, and translational nanotherapies for its treatment. Herein we hypothesize that non-proinflammatory nanomaterials sensitive to low pH or high reactive oxygen species (ROS) may serve as effective platforms for triggerable delivery of anti-atherosclerotic therapeutics in cellular and tissue microenvironments of inflammation. To demonstrate this hypothesis, an acid-labile material of acetalated β-cyclodextrin (β-CD) (Ac-bCD) and a ROS-sensitive β-CD material (Ox-bCD) were separately synthesized by chemical modification of β-CD, which were formed into responsive nanoparticles (NPs). Ac-bCD NP was rapidly hydrolyzed in mildly acidic buffers, while hydrolysis of Ox-bCD NP was selectively accelerated by H_2O_2. Using an anti-atherosclerotic drug rapamycin (RAP), we found stimuli-responsive release of therapeutic molecules from Ac-bCD and Ox-bCD nanotherapies. Compared with non-responsive poly(lactide-*co*-glycolide) (PLGA)-based NP, Ac-bCD and Ox-bCD NPs showed negligible inflammatory responses *in vitro* and *in vivo*. By endocytosis in cells and intracellularly releasing cargo molecules in macrophages, responsive nanotherapies effectively inhibited macrophage proliferation and suppressed foam cell formation. After intraperitoneal (*i.p.*) delivery in apolipoprotein E-deficient ($ApoE^{-/-}$) mice, fluorescence imaging showed accumulation of NPs in atherosclerotic plaques. Flow cytometry analysis indicated that the lymphatic translocation mediated by neutrophils and monocytes/macrophages may contribute to atherosclerosis targeting of *i.p.* administered NPs, in addition to targeting via the leaky blood vessels. Correspondingly, *i.p.* treatment with different nanotherapies afforded desirable efficacies. Particularly, both pH and ROS-responsive nanomedicines more remarkably delayed progression of atherosclerosis and significantly enhanced stability of atheromatous lesions, in comparison to non-responsive PLGA nanotherapy. Furthermore, responsive nanovehicles displayed good safety performance after long-term administration in mice. Consequently, for the first time our findings demonstrated the therapeutic advantages of nanomedicines responsive to mildly acidic or abnormally high ROS microenvironments for the treatment of atherosclerosis.

1. Introduction

Atherosclerosis remains the leading cause of morbidity and mortality in industrialized countries, with an increasing prevalence in developing countries. Therapeutic intervention is the most frequently employed strategy for the management of atherosclerosis [1]. Different therapeutics have been extensively utilized, clinically tested, or investigated in laboratories for

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atherosclerosis therapy [2–4]. However, all these drugs are generally administered orally, resulting in nonspecific distribution of drug molecules throughout the body, which is closely related to side effects, especially post long-term treatment. Accordingly, these dose-limiting adverse effects in other tissues or organs prohibit using higher doses that are required to achieve strong inhibitory effects. Furthermore, this systemic administration more often causes low concentration or poor retention of drug molecules in atherosclerotic plaques, which is responsible for the limited therapeutic outcome [5]. Targeted drug delivery by nanotechnology is a promising approach to address these issues [5–9]. Therapeutics, including small molecular drugs, peptides, proteins, and nucleic acids have been loaded into polymer nanoparticles (NPs), lipid NPs, or reconstituted high-density lipoprotein NPs for targeted therapy of atherosclerosis or its implications [10–17]. It has been demonstrated that NPs may target atherosclerotic plaques by translocation across the permeable atherosclerotic endothelium through the luminal side of the arterial vessels and/or transportation through the dysfunctional neovessels in the adventitia [5,18,19].

Nevertheless, there are still unmet demands for discovery and development of more effective and safe nanotherapies from the viewpoint of clinical applications. As well documented, atherosclerosis is a multifocal disease, and heterogeneous plaques with distinctively varied molecular and cellular components are randomly distributed along the aortic tree [20]. Consequently, treatment by either passive or active targeting based on a single molecular or cellular target is not an optimal regimen [2,5]. On the other hand, targeting the microenvironment shared by different diseases represents an intriguing approach, by using stimuli-responsive nanomaterials [21–24]. In this context, nanovehicles sensitive to various biochemical or physical signals have been extensively studied to spatiotemporally transport therapeutics to subcellular organelles or diseased sites [21–23,25–27]. Particularly, intensive studies have been conducted for therapy of tumors and gastrointestinal diseases by selective drug release from responsive delivery vehicles, and some promising preclinical results were obtained [28–30]. In addition, by using carriers with different chemical or topological structures, efficacies of responsive nanotherapies for the treatment of acute inflammatory diseases were demonstrated [31–36]. However, their effectiveness in the treatment of coronary heart disease remains to be established. As well documented, atherosclerosis is a chronic inflammatory disease, and inflammation plays a key role in coronary artery disease and other manifestations of atherosclerosis. Frequently, inflamed tissues are characterized by mildly acidic microenvironment [37–39]. In addition, oxidative stress induced by abnormally higher levels of reactive oxygen species (ROS) is intimately related to the pathogenesis and progression of atherosclerosis [40,41]. Accordingly, nanomedicines that can release drug molecules in response to lower pH or abnormally increased ROS have the potential of achieving additional therapeutic benefits.

On the other hand, nanocarriers derived from materials that may induce inflammatory reactions by themselves or their metabolites are undesirable for anti-atherosclerotic therapy, since they may exacerbate existing inflammation, especially upon long-term treatment. A case in point is polyester, a family of biodegradable polymers widely used for drug delivery, which may hydrolyze into acidic byproducts. Previous studies have evidenced that polyester such as polylactide and poly(lactide-*co*-glycolide) may activate inflammatory pathways *in vitro* and *in vivo* [42–45], leading to compromised therapeutic efficacy in cardiac dysfunction post acute myocardial infarction [42]. As a result, materials with minimal pro-inflammatory effects are desirable for constructing delivery systems to treat inflammation-associated coronary arterial disease.

Recently we have developed a series of pH- and ROS-responsive materials by functional modification of cyclodextrins [46–48]. Hydrolysis of these materials generates neutral products that cause minimal tissue inflammatory response after local administration in ocular mucosal tissue or intramuscular/intravenous injection of their NPs in rabbits or mice. Furthermore, NPs derived from these functional cyclodextrin materials can effectively load diverse therapeutics with distinctive chemical structures and molecular sizes [49–51]. Herein we hypothesize that pH- or ROS-responsive and biocompatible NPs based on cyclodextrin materials may serve as superior delivery vehicles over the PLGA counterpart for atherosclerosis treatment, by selectively releasing drug molecules in response to slightly acidic and abnormally high ROS microenvironments at inflammatory lesions. In addition, we attempt to elucidate which biochemical signal is more crucial for designing responsive nanotherapies. As a proof of concept, rapamycin (RAP) was used as a model drug, which has been found to be a promising and effective anti-atherosclerotic agent, while targeted delivery is required due to severe side effects after systemic administration [52–55].

2. Materials and methods

2.1. Materials

2-Methoxypropene (MP), β-cyclodextrin (β-CD), fluorescein diacetate (FDA), dichlorodihydrofluorescein diacetate (DCFH-DA), phorbol 12-myristate 13-acetate (PMA), carrageen, Oil Red O (ORO), and Toluidine Blue were purchased from Sigma-Aldrich (U.S.A.). Pyridinium *p*-toluene sulfonate (PTS), 1, 1′-carbonyldiimidazole (CDI), 4-dimethylaminopyridine (DMAP), and 4-(hydroxymethyl) phenylboronic acid pinacol ester (PBAP) were obtained from Acro Organics. Lecithin (from soybean) was obtained from Tokyo Chemical Industry Co, Ltd. (Tokyo, Japan). Poly(lactide-*co*-glycolide) [50:50] (PLGA) was supplied by Polysciences, Inc (U.S.A). 2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000 (DSPE-PEG) was provided by Corden Pharma (Switzerland). Rapamycin (RAP) was purchased from Beijing Huamaike Biotechnology. Cyanine 5 NHS ester (Cy5) was obtained from Lumiprobe (U.S.A.). Penicillin, streptomycin, and fetal bovine serum (FBS) were purchased from Gibco (U.S.A.). High-oxidized low-density lipoprotein (Ox-LDL) was purchased from Beijing Solarbio Science & Technology Co., Ltd. All other reagents are commercially available and used as received.

2.2. Synthesis of a pH-sensitive material from β-CD

The pH-responsive material of acetalated β-CD (Ac-bCD) was fabricated according to our previous study (Fig. S1) [46]. In brief, 4 mL MP (42 mmol) and 1 g β-CD (0.88 mmol) was dissolved in 10 mL DMSO, into which 16 mg PTS was added. After 3 h of acetalation at room temperature under magnetic stirring, 0.5 mL of triethylamine was added to terminate the reaction. The product was precipitated from water, washed by deionized water, collected by filtration, and then lyophilized to give rise to a white powder. The obtained material was characterized by ^{1}H NMR spectroscopy.