Biomaterials 105 (2016) 206–221

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

A superoxide dismutase/catalase mimetic nanomedicine for targeted therapy of inflammatory bowel disease

Qixiong Zhang [a, b], Hui Tao [a], Yongyao Lin [b], Ying Hu [a], Huijie An [a], Dinglin Zhang [b, c], Shibin Feng [d], Houyuan Hu [d], Ruibing Wang [e], Xiaohui Li [b, **], Jianxiang Zhang [a, *]

[a] Department of Pharmaceutics, College of Pharmacy, Third Military Medical University, 30 Gaotanyan Main St., Chongqing, 400038, China
[b] Institute of Materia Medica, College of Pharmacy, Third Military Medical University, 30 Gaotanyan Main St., Chongqing, 400038, China
[c] Department of Chemistry, College of Pharmacy, Third Military Medical University, Chongqing, 400038, China
[d] Department of Cardiology, Southwest Hospital, Third Military Medical University, Chongqing, 400038, China
[e] State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Taipa, Macau, China

ARTICLE INFO

Article history:
Received 2 June 2016
Received in revised form
2 August 2016
Accepted 5 August 2016
Available online 8 August 2016

Keywords:
Colitis
Reactive oxygen species
Responsive nanoparticles
Cyclodextrin
Drug delivery
Targeted therapy

ABSTRACT

Oxidative stress, resulting from excessive generation of reactive oxygen species (ROS), plays a pivotal role in the initiation and progression of inflammatory bowel disease (IBD). To develop an efficacious and safe nanotherapy against IBD, we designed and developed a superoxide dismutase/catalase mimetic nanomedicine comprising a hydrogen peroxide-eliminating nanomatrix and a free radical scavenger Tempol (Tpl). To this end, an oxidation-responsive β-cyclodextrin material (OxbCD) was synthesized, and a Tpl-loaded OxbCD nanoparticle (Tpl/OxbCD NP) was produced. Hydrolysis of OxbCD NP could be triggered by hydrogen peroxide, leading to on-demand release of loaded Tpl molecules from Tpl/OxbCD NP. OxbCD NP was able to efficiently accumulate in the inflamed colon in mice, thereby dramatically reducing nonspecific distribution after oral delivery. In three mouse colitis models, oral administration of Tpl/OxbCD NP notably mitigated manifestations relevant to colitis, and significantly suppressed expression of proinflammatory mediators, with the efficacy superior over free Tpl or a control nanomedicine based on poly(lactide-*co*-glycolide) (PLGA). Accordingly, by scavenging multiple components of ROS, Tpl/OxbCD NP may effectively reduce ulcerative colitis in mice, and it can be intensively developed as a translational nanomedicine for the management of IBD and other inflammatory diseases.

1. Introduction

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the gastrointestinal tract [1]. Crohn's disease and ulcerative colitis are two main clinical forms of IBD, and their prevalence adversely affects millions of patients around the world [2]. On the basis of different molecules and cells involved in the pathogenesis of IBD, diverse strategies and various drugs have been developed for IBD therapy [3]. Historically, nonspecific agents such as nonsteroidal anti-inflammatory drugs and corticosteroids as well as immunosuppressive and immunoregulatory agents were employed [1], which generally lead to significant side effects [4,5]. Discoveries of specific mechanisms responsible for abnormal and overactive intestinal inflammation in IBD have resulted in the development of biological therapeutics, including the currently approved anti-TNF agents and Natalizumab that can inhibit leukocyte trafficking to the gut [3]. Unfortunately, different limitations have been observed for these novel biologics. Besides poor response or developed intolerance in some patients [6], safety issues such as serious infections and malignancies occurred in some cases [7]. Accordingly, novel strategies and therapies need to be developed for effective treatment of IBD.

Reactive oxygen species (ROS) are a library of chemically reactive oxygen-containing molecules, resulting from the normal metabolism of oxygen. Whereas low levels of endogenously produced ROS are required to maintain normal cell functions by regulating oxygen homeostasis and cellular signaling [8], their over production is intimately related to the pathogenesis and development of inflammatory diseases [9,10]. Superoxide, peroxide, and hydroxyl radical are among the major components of ROS [11].

* Corresponding author.
** Corresponding author.
E-mail addresses: lpsh008@aliyun.com (X. Li), jxzhang1980@gmail.com (J. Zhang).

http://dx.doi.org/10.1016/j.biomaterials.2016.08.010
0142-9612/

Most intracellular ROS are derived from superoxide that is generated by the reduction of oxygen. Dismutation of superoxide by superoxide dismutase (SOD) generates hydrogen peroxide [12], which may be reduced to hydroxyl radical or decomposed into water by catalase. Both experimental and clinical evidence has demonstrated that overproduced ROS, by infiltrated inflammatory cells in the intestinal mucosa, may amplify the inflammatory response, trigger mucosal injury, and accelerate mucosal ulceration in the pathogenesis of IBD [11,13–15]. It has been found that mucosal ROS concentrations are 10–100 times higher in patients suffering from IBD [16,17]. Studies in animal models and patients indicated that treatment with either antioxidants or free radical scavengers may mitigate Crohn's disease and colitis [18–20]. Nevertheless, only limited success has been achieved thus far due to multiple reasons. First, the instability of many therapeutics in the harsh, acidic, and enzyme-rich environment encountered in the gastrointestinal tract may compromise their pharmacological activities and impair their *in vivo* performance. Second, nonspecific drug distribution and rapid clearance generally result in lower drug concentrations at diseased sites concomitant with undesirable drug distribution in normal tissues, which eventually compromises efficacy while generating side effects [21]. Third, most of previously investigated therapeutic regimens against oxidative stress in IBD eliminate or decrease only one or two components of ROS. Consequently, innovative antioxidant strategies are urgently required for the effective prevention and resolution of intestinal inflammation in IBD.

Recently, nanomedicinal approaches have been demonstrated to have great potential for diagnosis, prevention, and therapy of various diseases [22]. In particular, considerable success has been achieved in tumor therapy, with tens of nanomedicines approved for clinical use, and more than one hundred nanotherapeutics are currently in clinical trials [23]. For the treatment of gastrointestinal diseases, nanoparticles (NPs) also showed special advantages, such as protecting payload from destabilization or hydrolysis, improving bioavailability, and increasing drug release/retention at diseased sites [24–27]. Besides, recent studies in animal models suggested that NPs either physically or chemically loaded with different agents displayed notably improved therapeutic efficacy in IBD and IBD-associated diseases [28–34], largely resulting from localized delivery of therapeutics to diseased intestinal tissues [25,35–38]. However, for the clinical translation of currently developed nanomedicines for IBD therapy, there is still great challenge in cost-efficient mass synthesis of delivery systems with well-tailored structures, beneficial responsive and other physicochemical properties, desirable targeting capability, excellent *in vivo* biocompatibility, reproducible production, and good quality control [37].

In view of the important roles of SOD and catalase in eliminating ROS under physiological conditions, herein we hypothesize that nanomedicines, functionally mimicking SOD and catalase, may serve as effective therapeutics for IBD (Fig. 1A-B). As a conceptual proof study and with the aim to develop a tanslationable nanotherapy, we designed a nanosystem based on Tempol (Tpl) and a biocompatible β-cyclodextrin-derived material (Tpl/OxbCD NP). The molecular payload Tpl of this nanomedicine serves as a SOD-mimetic [39], while the nanocontainer is mainly composed of a hydrogen peroxide-eliminating material OxbCD that functions as a catalase-mimicker. In the existence of hydrogen peroxide, OxbCD may be hydrolyzed into water soluble products including β-cyclodextrin, pinacol borate, and *p*-hydroxylmethylphenol, thereby leading to elimination of hydrogen peroxide, disruption of the nanocontainer, and release of Tpl molecules. This SOD/catalase mimetic nanotherapy of Tpl/OxbCD NP can effectively scavenge multiple components of ROS such as superoxide, hydroxyl radical, and hydrogen peroxide. Post oral delivery, this newly developed nanomedicine, which is stable in the gastrointestinal tract, can target inflammatory sites due to increased epithelial permeability and enhanced physicochemical binding interactions. Upon triggering by abnormally elevated ROS levels, this SOD/catalase mimetic nanomedicine can selectively release Tpl molecules in inflamed intestinal tissues. *In vivo* studies showed that the newly developed nanomedicine exhibited robust therapeutic benefits and good safety profiles in mice with acute or chronic colitis induced by either dextran sodium sulfate (DSS) or 2,4,6-trinitro benzene sulfonic acid (TNBS).

2. Materials and methods

2.1. Materials

Tempol (Tpl), 4-(hydroxymethyl) phenylboronic acid pinacol ester (PBAP), N, N′-carbonyldiimidazole (CDI), 4-dimethylaminopyridine (DMAP), anhydrous dichloromethane (DCM), 2, 4, 6-trinitro benzene sulfonic acid (TNBS), and 4′, 6-diamidino-2-phenylindole (DAPI) were purchased from Sigma (St. Louis, USA). β-Cyclodextrin (β-CD) was supplied by Zhiyuan Biotechnology Co., Ltd (Shandong Binzhou, China). N-(Carbonyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine (DSPE-PEG) was obtained from Avanti Polar Lipids Inc (USA). Lecithin was supplied by Tokyo Chemical Industry Co., Ltd (Tokyo, Japan). Poly(lactide-*co*-glycolide) (PLGA, 50:50, with intrinsic viscosity of 0.50–0.65) was purchased from Polysciences Inc (USA). Cy5 NHS ester and Cy7.5 NHS ester were obtained from Lumiprobe (USA). Dextran sulfate sodium (DSS, 35000 Da) was supplied by MP Biomedical (USA). The APC-labeled anti-mouse EpCAM (CD326) antibody was purchased from eBioscience (USA). PE-labeled anti-mouse CD98 antibody was purchased from Biolegend (USA). All ELISA kits and oxidative stress-related kits were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). BCA protein assay kit was purchased from Beyotime Biotechnology (China). All the other reagents are commercially available and used as received.

2.2. Synthesis and characterization of ROS-responsive β-CD material (OxbCD)

A ROS-responsive material OxbCD was synthesized according to our previously established methods [40]. Specifically, 5.55 g PBAP was dissolved in 36 mL of anhydrous DCM, into which 7.65 g CDI was added and magnetically stirred for 1 h at room temperature. After 40 mL of DCM was added into the mixture, it was washed with deionized water three times. The organic phase was further rinsed with saturated NaCl solution, and dried over Na_2SO_4. Then CDI-activated PBAP was obtained by drying under vacuum. Subsequently, 1.52 g activated PBAP and 250 mg β-CD were co-dissolved in 20 mL of anhydrous dimethyl sulfoxide. After 0.8 g of DMAP was added, reaction was carried out overnight at 25 °C. The final product was collected by precipitation from 80 mL of deionized water, followed by centrifugation. After thorough rinsing with deionized water, the sample was lyophilized to give a white powder. The obtained OxbCD was characterized by ^{1}H NMR and Fourier transform-infrared (FT-IR) spectroscopy.

2.3. Fabrication of nanoparticles by a nanoprecipitation/self-assembly method

The Tpl-loaded OxbCD nanoparticle (Tpl/OxbCD NP) was prepared by a modified nanoprecipitation/self-assembly method [40]. Briefly, 20 mg Tpl and 50 mg OxbCD were dissolved in 2 mL of methanol. The obtained solution was added dropwise into 10 mL of