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Development of an oral nanotherapeutics using redox nanoparticles for treatment of colitis-associated colon cancer

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ABSTRACT

Oral chemotherapy is the preferred treatment for colon cancer. However, this strategy faces many challenges, including instability in the gastrointestinal (GI) tract, insufficient bioavailability, low tumor targeting, and severe adverse effects. In this study, we designed a novel redox nanoparticle (RNP^O) that is an ideal oral therapeutics for colitis-associated colon cancer treatment. RNP^O possesses nitroxide radicals in the core, which act as reactive oxygen species (ROS) scavengers. Orally administered RNP^O highly accumulated in colonic mucosa, and specifically internalized in cancer tissues, but less in normal tissues. Despite of long-term oral administration of RNP^O, no noticeable toxicities were observed in major organs of mice. Because RNP^O effectively scavenged ROS, it significantly suppressed tumor growth after accumulation at tumor sites. Combination of RNP^O with the conventional chemotherapy, irinotecan, led to remarkably improved therapeutic efficacy and effectively suppressed its adverse effects on GI tract. Therefore, RNP^O is promising oral nanotherapeutics for cancer therapies.

1. Introduction

Inflammatory bowel disease (IBD), which includes chronic gastrointestinal (GI) disorders such as Crohn's disease (CD) and ulcerative colitis (UC), affects millions of patients worldwide [1–4]. After 30 years of living with these diseases, 18–20% of UC and 8% of CD patients develop colitis-associated colon cancer (CAC), the third most common malignancy and one of the major causes of cancer-related death [5,6]. In IBD patients, the increasing of reactive oxygen species (ROS) causes oxidative stress and oxidative cellular damage promoting carcinogenesis [7,8]. It has been reported that antioxidants such as *N*-acetylcysteine and resveratrol inhibited CAC development [9,10]. While oral administration of drugs are preferred by patient due to its convenience and compliance, these low-molecular-weight (LMW) compounds are not always effective due to nonspecific drug distribution, low retention in the GI tract, and absorption in the bloodstream, causing undesired adverse effects in the entire body. On the other hand, chemotherapy using 5-fluorouracil (5-FU) or irinotecan (Iri) has been used alone or in combination with other drugs as the first-line therapeutic agents for colorectal cancer [11,12]. However, these anticancer drugs are insufficient bioavailability and low tumor targeting. Furthermore, patients treated with these chemotherapeutic agents suffer from severe adverse effects such as mucositis and diarrhea, which limits the dose intensification and compromises efficacy [13].

Nanotechnology has enabled significant advances in the areas of cancer diagnosis and therapy [14–16]. Though a number of nanoparticle-drug combinations are assessed in preclinical or clinical applications, most of delivery systems are intravenously injectable formulations and are incapable of oral administration [17,18]. On the other hand, it has been reported that nanocomposites such as silver nanoparticle for therapeutics itself exhibits the undesired toxicity on the GI tract after repeated oral administration [19,20]. Recently, we have developed an oral nanotherapy using a redox nanoparticle (RNP^O) for suppressing

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inflammation in mice with colitis [21] and indomethacin-induced small intestinal inflammation [22]. RNP^O was prepared by self-assembly of methoxy-poly(ethylene glycol)-*b*-poly(4-[2,2,6,6-tetramethylpiperidine-1-oxyl]oxymethylstyrene)] (MeO-PEG-*b*-PMOT), which is an amphiphilic block copolymer with stable nitroxide radicals in a hydrophobic segment as a side chain via an ether linkage (Fig. 1A). The size of RNP^O is approximately 40 nm in diameter, with a remarkably narrow distribution (Fig. 1B) and extremely high colloidal stability owing to the PEG shell layer. As shown in Fig. 1C, RNP^O is stable and maintains micelle form under physiological conditions without aggregation. This stable character improves accumulation tendency of RNP^O to colonic mucosa, but not commercially available polystyrene particles. Furthermore, these 40 nm particles prevent the uptake into bloodstream via mesentery. Along with these characteristics, we have confirmed that RNP^O effectively scavenges ROS to result in significant suppression of inflammation in mice with colitis [21]. Suppression of inflammation in the tumor microenvironments is reported to work as suppressor of tumor progression and resistance against chemotherapy [23]. Notably, we have confirmed that RNP^O did not cause any disturbance to the population of intestinal bacteria [24]. Based on these characteristics of RNP^O, we proposed that it would be a suitable oral therapeutics for cancer. Thus, it is interesting to apply RNP^O as a novel oral therapeutics for treatment of colon cancer.

In this study, we used azoxymethane (AOM) and dextran sodium sulfate (DSS) to chemically induced CAC in mice, and we confirmed the efficacy of oral RNP^O as a nanomedicine and combination therapy. No blood absorption and non-toxicity of RNP^O were observed despite of long-term oral administration, which improves accumulation in colon region and prevents undesired adverse effects to entire body. We also found that orally administered RNP^O tends to internalize in colon cancer cells, but not normal colon cells, indicating the extremely low adverse effects of this oral nanotherapeutics. Oral administration of RNP^O effectively suppressed inflammation in the colon region, resulting in both high protective and therapeutic effects against CAC development. It is interesting to note that when RNP^O was combined with conventional chemotherapy, the therapeutic effect on CAC was significantly enhanced, retaining low adverse effects of the chemotherapy on the GI tract.

2. Materials and methods

2.1. Preparation of RNP^O

RNP^O was prepared by a self-assembling MeO-PEG-*b*-PMOT block copolymer, as previously reported [21,25]. Briefly, methoxy-poly(ethylene glycol)-*b*-poly(-chloromethylstyrene) (MeO-PEG-*b*-PCMS) was synthesized by the radical telomerization of chloromethylstyrene (CMS; Seimi Chemical Co., Ltd., Kanagawa, Japan) using methoxy-poly(ethylene glycol)-sulphanyl (MeO-PEG-SH; NOF Corporation Co., Ltd., Tokyo, Japan; Mn = 5000) as a telogen (the degree of polymerization of CMS = 16 units). The chloromethyl groups were converted to TEMPOs via a Williamson ether synthesis of benzyl chloride in the MeO-PEG-*b*-PCMS block copolymer with the alkoxide of 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPOL; Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), as previously reported (the extent of TEMPO modification = 85%). RNP^O was prepared from MeO-PEG-*b*-PMOT using a dialysis method.

2.2. Cell lines and cultures

The mouse colorectal carcinoma cell line C-26 (RCB2657) was obtained from Riken BioResource Center (Riken Tsukuba Institute, Ibaraki, Japan). C-26 cells were grown in Dulbecco's modified eagle medium (DMEM; Sigma–Aldrich, St. Louis, MO) containing 10% fetal bovine serum (Sigma–Aldrich, St. Louis, MO), and 1% antibiotics (penicillin/streptomycin/neomycin; Invitrogen, Carlsbad, CA) in a humidified atmosphere of 5% CO_2 at 37 °C.

2.3. Cellular uptake of RNP^O in vitro

The experiment was carried out using rhodamine-labeled RNP^O (Rho-RNP^O) to analyze the cellular uptake of these nanoparticles by fluorescent confocal microscope. Rho-RNP^O was prepared via a thiourethane bond between MeO-PEG-*b*-PMOT

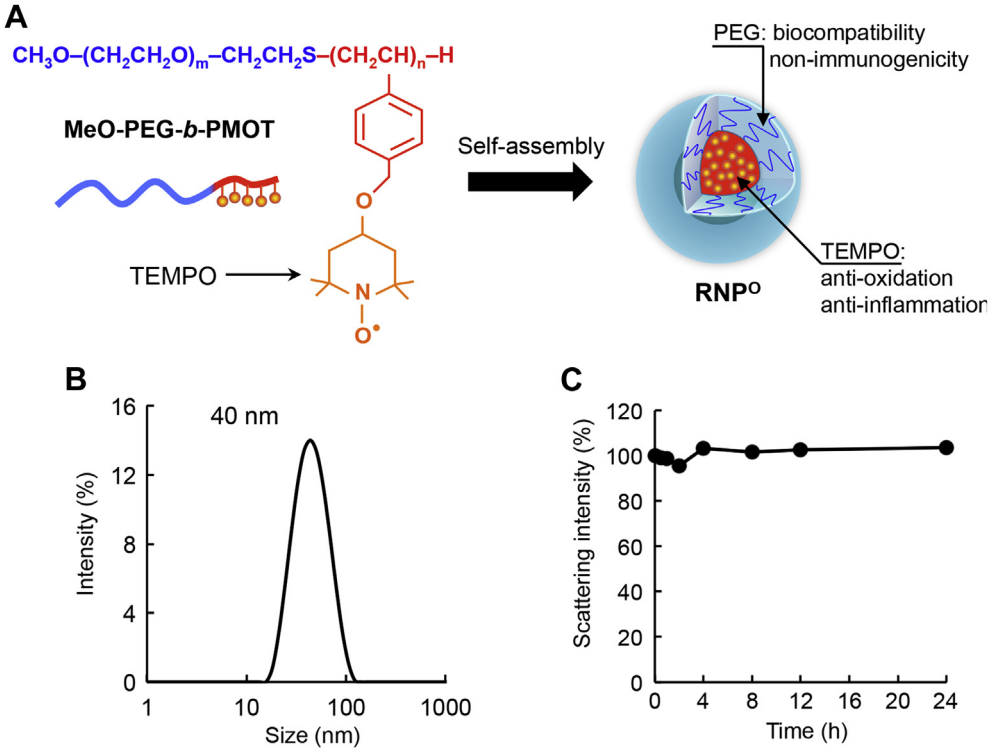

Fig. 1. Schematic illustration of RNP^O and its characteristics. (A) RNP^O was prepared by self-assembly of a poly(ethylene glycol)-*b*-poly(4-methylstyrene) block copolymer possessing nitroxide radical TEMPO moieties [21]. (B) The size of RNP^O and (C) the stability of RNP^O under physiological conditions (PBS pH 7.4, 10% FBS) were measured by dynamic light scattering using a Zetasizer Nano ZS (Malvern Instruments, Ltd., Malvern, UK).