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Fluorocoxib A loaded nanoparticles enable targeted visualization of cyclooxygenase-2 in inflammation and cancer

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ABSTRACT

Cyclooxygenase-2 (COX-2) is expressed in virtually all solid tumors and its overexpression is a hallmark of inflammation. Thus, it is a potentially powerful biomarker for the early clinical detection of inflammatory disease and human cancers. We report a reactive oxygen species (ROS) responsive micellar nanoparticle, PPS-b-POEGA, that solubilizes the first fluorescent COX-2-selective inhibitor fluorocoxib A (FA) for COX-2 visualization in vivo. Pharmacokinetics and biodistribution of FA-PPS-b-POEGA nanoparticles (FA-NPs) were assessed after a fully-aqueous intravenous (i.v.) administration in wild-type mice and revealed 4–8 h post-injection as an optimal fluorescent imaging window. Carrageenan-induced inflammation in the rat and mouse footpads and 1483 HNSCC tumor xenografts were successfully visualized by FA-NPs with fluorescence up to 10-fold higher than that of normal tissues. The targeted binding of the FA cargo was blocked by pretreatment with the COX-2 inhibitor indomethacin, confirming COX-2-specific binding and local retention of FA at pathological sites. Our collective data indicate that FA-NPs are the first i.v.-ready FA formulation, provide high signal-to-noise in inflamed, premalignant, and malignant tissues, and will uniquely enable clinical translation of the poorly water-soluble FA compound.

1. Introduction

Cyclooxygenases (COXs) are important biological mediators of inflammation that catalyze the biotransformation of arachidonic acid into prostaglandins and thromboxane [1]. Most normal tissues express the COX-1 isoform, which performs housekeeping functions, such as maintenance of vascular tone, control of hemostasis, and cytoprotection of the gastric mucosa [2]. In contrast, the inducible COX-2 isoform is overexpressed in inflammation, where it modulates edema and pain, and in neoplastic diseases, where it potentiates tumor growth and metastasis [3]. Overexpression of COX-2 is an early event in carcinogenesis, and it plays a vital role in cancer progression [4], suggesting that it is a useful biomarker for both early- and late-stage cancer detection. Moreover, COX-2 inhibitors have been shown to be effective adjuvant chemotherapeutic agents in some cancers [5,6]. Therefore, COX-2 is an ideal candidate for targeted visualization of inflammatory disease and a broad spectrum of human cancers. To this end, we discovered fluorocoxib A (FA), a fluorescent 5-carboxy-X-rhodamine- (5-ROX)-labeled COX-2-selective inhibitor, to visualize COX-2 in inflamed or cancerous tissues [7].

Clinical translation of FA has the potential to address the significant, unmet need for techniques that enable earlier detection of cancers of the skin, colon, esophagus, bladder, and oropharynx [7–10]. For example, five year survival for colorectal cancer is 90% if diagnosed while it is still localized, but falls to 68% for regional disease, and just 10% for disease with distant metastases [11]. Moreover, miss rates for colorectal neoplastic polyps can be as high

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as 28% by traditional white-light colonoscopy [12]. Likewise, cancers of the esophagus, bladder, and oropharynx show higher survival rates and better prognoses when detected early (before regional spreading and metastases) [13–15]. Despite the clear need, translation of optical imaging agents from basic research tools to clinically relevant contrast agents remains challenging [16]. The Molecular Imaging and Contrast Agents Database lists 1444 agents as of June 2013. Of these, only 119 agents have been FDA approved, and only 4 are optical imaging agents (all Indocyanine Green and Fluorescein derivatives).

Attempts to translate FA to the clinic have been hampered by its lack of solubility in aqueous solutions appropriate for human administration. All previous administrations of FA have been in 100% dimethyl sulfoxide (DMSO) or a mixed solvent consisting of DMSO (16%)/ethanol (33%)/propylene glycol (17%)/warm sterile saline (34%, 37.5 °C), that are not appropriate for human applications, especially by i.v. administration. Thus, a new formulation strategy is necessary before the clinical potential of FA can be realized. Many promising small molecule drugs and imaging agents, such as FA, suffer from extreme hydrophobicity, limiting use in an injectable form. Polymeric nanoparticles provide a promising approach for solubilizing and altering the pharmacokinetics of such small molecules in vivo [17,18]. For example, amphiphilic diblock polymers can be designed to self-assemble into micellar nanoparticles that enable solubilization of hydrophobic compounds by sequestering them into a hydrophobic core surrounded by a hydrated corona. Utilization of inert, hydrophilic macromolecules such as poly(ethylene glycol) (PEG) to form the corona enhances nanoparticle stealth, reducing opsonization and rate of clearance by the mononuclear phagocyte system (MPS) [19–22]. Ideal nanoparticle formulations have a hydrodynamic diameter greater than ~10 nm, which avoids rapid renal clearance, and fall into a size range (approximately 20–200 nm) that enhances passive targeting to cancer and inflammation by the enhanced permeability and retention (EPR) effect [23,24]. Material responsiveness to environmental cues, such as changes in pH, enzyme activity, and reactive oxygen species (ROS), can then be leveraged to trigger cargo release within these tissues [25–30]. Importantly, micellar systems are being tested clinically and in some cases (e.g. Genexol-PM) have made it through clinical trials to become approved for use in humans, supporting the potential translatability of the proposed approach [31].

Herein, we report the synthesis and characterization of a novel diblock polymer that self-assembles into water-soluble micellar nanoparticles (NPs) that efficiently encapsulate/solubilize FA. We describe the pharmacokinetics and biodistribution of FA-NPs and validate COX-2-specific delivery and binding of FA, released from FA-NPs, in vivo by blocking the COX-2 active site through pretreatment with a high-affinity inhibitor. These studies were designed to demonstrate that formulation into NPs enables i.v. use and clinical translation of FA for optical diagnosis of inflammatory and neoplastic diseases.

2. Materials and methods

2.1. Polymer synthesis and characterization

We synthesized a diblock polymer-based micellar nanoformulation of FA (Fig. 1). The chemical structure and photophysical properties of FA are described in Fig. 1A. The diblock polymer, poly(propylene sulfide)$_{106}$-*b*-poly[oligo(ethylene glycol)$_9$ methyl ether acrylate]$_{17}$ (PPS$_{106}$-*b*-POEGA$_{17}$), was synthesized by a combination of anionic and reversible addition-fragmentation chain-transfer (RAFT) polymerization (Fig. 1B) as described below.

2.1.1. Synthesis of hydroxyl end-functionalized poly(propylene sulfide) (PPS$_{106}$-OH)

Poly(propylene sulfide) was prepared by anionic polymerization of propylene sulfide using DBU/1-butanethiol as an initiator and subsequently end-capped with 2-iodoethanol to yield a terminal hydroxyl group. 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (6.0 mmol, 0.897 mL) was dissolved in dry tetrahydrofuran (THF) (15 mL) in a dried and nitrogen flushed 50 mL round bottom flask and degassed for 30 min before lowering the reaction temperature to 0 °C. 1-Butanethiol (2.0 mmol, 0.14 mL) in THF (5 mL) was added drop wise to the flask and allowed to react for 30 min. Later, freshly distilled and degassed propylene sulfide (120 mmol, 9.387 mL) monomer was added to the reaction mixture, and the temperature was maintained at 0 °C for 2 h. The reaction was quenched by addition of 2-iodoethanol (6.0 mmol, 1.03 g) and stirred overnight at room temperature [32]. After stirring overnight, the polymer solution was filtered to remove precipitated salt and further purified by three precipitations into cold methanol before vacuum-drying to yield a colorless viscous polymer. ^{1}H NMR (400 MHz; $CDCl_3$, δ): 1.3–1.4 (s, CH_3), 2.5–2.8 (s, –CH), 2.8–3.1 (s, CH_2), 3.72 (t, CH_2–OH). (PPS$_{106}$–OH, $M_{n,\ GPC}$ = 7800 g/mol, PDI = 1.4).

2.1.2. Synthesis of poly(propylene sulfide)-4-cyano-4-(ethylsulfanylthiocarbonyl) sulfanylpentanoic acid (PPS$_{106}$-ECT RAFT macro-CTA)

N,N′-Dicyclohexylcarbodiimide (DCC) (0.248 g, 1.2 mmol) was added to a solution of 4-cyano-4-(ethylsulfanylthiocarbonyl) sulfanylpentanoic acid (ECT) (2, 0.314 g, 1.2 mmol), PPS$_{106}$-OH (3.12 g, 0.4 mmol), and 4-dimethylaminopyridine (DMAP) (0.015 g, 0.12 mmol) in anhydrous dichloromethane (20 mL) at 0 °C [33]. After stirring at room temperature for 24 h, the reaction mixture was filtered to remove precipitated dicyclohexyl urea and concentrated under vacuum. The crude reaction mixture was first purified by dialysis against dichloromethane for 24 h to remove free ECT, further purified through double precipitation into cold ethanol, and characterized by ^{1}H NMR spectroscopy (Fig. S1). ^{1}H NMR (400 MHz; $CDCl_3$, δ): 1.35 (t, 3H, –S–CH_2–CH_3), 1.3–1.4 (s, 3H, CH_3), 1.85 (s–C(CN)–CH_3), 2.4–2.67 (m, –CH_2–CH_2–S), 2.5–2.8 (broad s, S–CH), 2.8–3.1 (broad s, 2H, CH_2), 3.42 (q, –S–CH_2–CH_3), 3.8 (t, –OCH_2-CH_2). (PPS$_{106}$-ECT, $M_{n,\ GPC}$ = 8100 g/mol, PDI = 1.4).

2.1.3. Synthesis of poly(propylene sulfide)-b-poly[oligo(ethylene glycol) methyl ether acrylate] (PPS$_{106}$-b-POEGA$_{17}$) diblock copolymer

The diblock copolymer PPS$_{106}$-*b*-POEGA$_{17}$ was synthesized by RAFT polymerization from a PPS$_{106}$-ECT macro-chain transfer agent (macro-CTA) using azobisisobutyronitrile (AIBN) at a 10:1 (macro-CTA:AIBN) molar ratio as the radical initiator. In a dry round bottom flask (10 mL), PPS$_{106}$-ECT (0.743 g, 0.095 mmol, Mn = 8200 Da), OEGA (1.21 mL, 2.86 mmol), and AIBN (1.56 mg, 9.5 μmol) in dioxane (5 mL) were degassed by nitrogen purging for 30 min. The reaction was allowed to proceed for 24 h at 70 °C. The reaction product was dialyzed against methanol for 24 h, dried under vacuum to yield a purified, milky white polymer, and characterized by ^{1}H NMR spectroscopy (Figure S2). ^{1}H NMR (400 MHz; $CDCl_3$, δ): 1.35 (t, 3H, –S–CH_2–CH_3), 1.3–1.4 (s, 3H, CH_3), 1.85 (s–C(CN)–CH_3), 2.4–2.67 (m, –CH_2–CH_2–S), 2.5–2.8 (broad s, S–CH), 2.8–3.1 (broad s, 2H, CH_2), 3.42 (q, –S–CH_2–CH_3), 3.68 (m, –OCH_2-CH_2), 3.8 (t, –OCH_2-CH_2). (PPS$_{106}$-*b*-POEGA$_{17}$, $M_{n,NMR}$ = 16,004 g/mol).

2.1.4. Polymer characterization

^{1}H NMR spectra were collected for all polymers in $CDCL_3$ on a Brüker 400 MHz spectrometer. Molecular weights (Mn), polydispersities (PDI), and compositions were determined by either ^{1}H NMR (Fig. S3) or gel permeation chromatography (GPC) (Agilent