Journal of Controlled Release 263 (2017) 46–56

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Silk-elastinlike protein polymers enhance the efficacy of a therapeutic glycosaminoglycan for prophylactic treatment of radiation-induced proctitis

Mark Martin Jensen [a,b], Wanjian Jia [c], Kyle J. Isaacson [a,b], Austin Schults [c], Joseph Cappello [d], Glenn D. Prestwich [e], Siam Oottamasathien [c,f,*], Hamidreza Ghandehari [a,b,d,**]

[a] Department of Bioengineering, University of Utah, Salt Lake City, UT 84112, USA
[b] Utah Center for Nanomedicine, Nano Institute of Utah, University of Utah, Salt Lake City, UT 84112, USA
[c] Division of Urology, Section of Pediatric Urology, University of Utah, Salt Lake City, UT 84113, USA
[d] Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Salt Lake City, UT 84112, USA
[e] Department of Medicinal Chemistry, University of Utah, Salt Lake City, UT 84112, USA
[f] Department of Surgery and Division of Pediatric Urology, Primary Children's Hospital, Salt Lake City, UT 84113, USA

ARTICLE INFO

Article history:
Received 10 December 2016
Received in revised form 15 February 2017
Accepted 19 February 2017
Available online 21 February 2017

Keywords:
Semi-synthetic glycosaminoglycan (GAG)
Radiation-induced proctitis
Silk-elastinlike protein polymer (SELP)
Radiation Enteropathy
Hyaluronic acid
Colitis

ABSTRACT

Radiation-induced proctitis (RIP) is the most common clinical adverse effect for patients receiving radiotherapy as part of the standard course of treatment for ovarian, prostate, colon, and bladder cancers. RIP limits radiation dosage, interrupts treatment, and lowers patients' quality of life. A prophylactic treatment that protects the gastrointestinal tract from deleterious effects of radiotherapy will significantly improve patient quality of life and may allow for higher and more regular doses of radiation therapy. Semi-synthetic glycosaminoglycan (GAG), generated from the sulfation of hyaluronic acid, are anti-inflammatory but have difficulty achieving therapeutic levels in many tissues. To enhance the delivery of GAG, we created an in situ gelling rectal delivery system using silk-elastinlike protein polymers (SELPs). Using solutions of SELP 815K (which contains 6 repeats of blocks comprised of 8 silk-like units, 15 elastin-like units, and 1 lysine-substituted elastin-like unit) with GAG GM-0111, we created an injectable delivery platform that transitioned in <5 min from a liquid at room temperature to a hydrogel at body temperature. The hydrogels released 50% of their payload within 30 min and enhanced the accumulation of GAG in the rectum compared to traditional enema-based delivery. Using a murine model of radiation-induced proctitis, the prophylactic delivery of a single dose of GAG from a SELP matrix administered prior to irradiation significantly reduced radiation-induced pain after 3, 7, and 21 days by 53 ± 4%, 47 ± 10%, and 12 ± 6%, respectively. Matrix-mediated delivery of GAG by SELP represents an innovative method for more effective treatment of RIP and promises to improve quality of life of cancer patients by allowing higher radiotherapy doses with improved safety.

1. Introduction

Radiation-induced proctitis (RIP) is the most common clinical issue for patients receiving radiotherapy as part of the standard course of treatment for ovarian, prostate, colon, and bladder cancers and limits radiation dosages, interrupts treatment, and reduces patient quality of life [1]. >200.000 cancer patients receive abdominal or pelvic radiation therapy annually, and the number of cancer survivors with post-radiation intestinal dysfunction continues to grow [1,2]. Acute RIP occurs in >75% of patients receiving radiotherapy for prostate cancer and progresses to debilitating chronic RIP in 5–10% of cases [3,4]. RIP is manifested by bleeding, pain, abdominal cramping, mucoid discharge, diarrhea, fecal urgency, and tenesmus [5].

During radiotherapy for prostate, cervical, ovarian, and bladder cancer, portions of the colon and rectum often fall within the radiation field due to anatomical proximity. Even with image-guided placement of the beams and protective shielding, irradiation of the cancerous tissue without also irradiating the rectum or other sensitive organs is not feasible [6–9]. The exact etiology and pathophysiology of RIP is not completely understood, but it is widely accepted that radiation-induced damage to lipids and DNA triggers mucosal atrophy, submucosal edema, and

* Correspondence to: S. Oottamasathien, 36 S. Wasatch Dr., Salt Lake City, UT 84112-5001, USA.
** Correspondence to: H. Ghandehari, 100 North, Mario Capecchi Dr., Suite 2200, Salt Lake City, UT 84113, USA.
E-mail addresses: siam.oottamasathien@hsc.utah.edu (S. Oottamasathien), hamid.ghandehari@utah.edu (H. Ghandehari).

http://dx.doi.org/10.1016/j.jconrel.2017.02.025
0168-3659/

inflammation that cause RIP [10]. Symptoms of RIP can emerge immediately or months after radiotherapy and can persist for 20 years post-diagnosis [11]. Chronic RIP can lead to life-threatening complications including fistula formation, sepsis, perforation, and internal bleeding [12]. A prophylactic treatment that protects the gastrointestinal tract from the deleterious effects of radiotherapy will improve patient quality of life during and after treatment [13–15] and may allow for higher doses of radiation to be administered more regularly, leading to improved clinical outcome [15,16]. No effective clinical prophylactic treatment options exist for the prevention of RIP in spite of its prevalence and clinical significance. Current treatments for RIP are reactionary and typically administered only after the onset of RIP. Previously investigated treatments such as sucralfate [17], 5-amino-salcylic acid [18], short-chain fatty acids [19], sodium butyrate [20], hydrocortisone [21], vitamin E [22], epinephrine [23], hyaluronic acid [24], and topical application of formalin [25] frequently fail to make significant improvement in patient quality of life and have even been shown to exacerbate the disease in rare cases [26]. After the failure of pharmacological treatments, physicians utilize surgical interventions, including laser ablation [27,28], electrocauterization [29], sclerotic injections [30], argon plasma coagulation [31], and radiofrequency ablation [32], all of which carry significant risks of morbidity and mortality. These surgical interventions frequently increase rectal pain, diarrhea, tenesmus, ulcers, fistula, rectal stenosis, and anal strictures [25,33]. What is needed is a non-invasive prophylactic treatment that prevents the onset of RIP or ameliorates the symptoms to levels that do not deleteriously impact patient quality of life.

Novel chemically sulfated glycosaminoglycans (GAG) (Fig. 1A) have been shown to elicit potent preventative effects against mucosal inflammation [34–36]. These highly anionic polysaccharides are synthesized by chemical sulfation of hyaluronic acid [37]. Previous data have shown that a leading synthetic GAG, GM-0111, is far more effective at preventing inflammation when compared to hyaluronic acid and other clinically available anti-inflammatory agents, and significantly decrease mast cell numbers in the inflamed tissue, making it a promising agent for addressing the inflammation and pain associated with radiation-induced proctitis [34–36,38]. However, a challenge of GM-0111 is achieving sufficient residence time to allow penetration into the surrounding tissues in therapeutically effective concentrations.

Silk-elastinlike protein polymers (SELPs) combine the strength of *Bombyx mori* (silkworm) silk [39] (GAGAGS) and the flexibility of mammalian elastin (GVGVP) [40] via genetic recombination. SELP-815K (which contains 6 repeats of blocks comprised of 8 silk-like units, 15 elastin-like units, and 1 lysine-substituted elastin-like unit (Fig. 1B), exists as an aqueous solution at room temperature and, depending on composition and sequence, can rapidly form a solid hydrogel matrix at body temperature, enabling sustained delivery of therapeutics over an extended period of time [41–43]. The unique recombinant synthesis of SELPs provides a high degree of control over polymer sequence and molecular weight which in turn allows precise control of hydrogel physicochemical properties [44,45]. These attributes include control over gelation kinetics and viscosity, bioactive agent release, biodegradation and elimination [43–45].

We hypothesized that SELP can be used to enhance GAG delivery and reduce the required frequency of administration, improve the pharmacokinetic profile, reduce systemic side effects and increase concentration at the site of action. The most efficient method to deliver therapy to colorectal tissue is via rectal administration of a suppository or enema. Here we developed an in situ gelling enema to enhance the delivery of GAG to colorectal tissue. Entering as a liquid, an in situ gelling enema is easy to administer and can coat the wall of the rectum prior to transitioning into a solid hydrogel. The intimate contact of the hydrogel with the wall of the rectum could enhance the accumulation of GAG by increasing the residence time of the GAG and promoting its diffusion into the lumen of the rectum. We investigated the use of varying concentrations of SELP 815K that are known to be injectable and transition to solid hydrogels at body temperature, for their ability to deliver GAG to the rectum.

2. Materials and methods

2.1. Materials

SELP 815K was synthesized, characterized, and validated as previously described, and was sheared in accordance to enhance the homogeneity and improve material properties [42,46]. GAG GM-0111 and poly(ethylene glycol) (PEG) suppositories were provided by GlycoMira Therapeutics, Inc. (Salt Lake City, UT).

2.2. In vitro release

The release of GAG from a SELP matrix was quantified using an Azure A base colorimetric assay. Samples of SELP 12% and GAG GM-0111 (lyophilized form) were mixed to create a 100 mg/ml GAG in SELP (final SELP concentrations of 4 wt% or 11 wt%) solution and loaded into 500 μl insulin syringes (Becton Dickenson and Company, Franklin Lakes, NJ), and then incubated overnight at 37 °C. The tip was severed from the syringe and the gel sectioned into 20 μl disks. The mass of the disks was measured and recorded. Samples were placed into test tubes (Bioexpress, Kaysville, UT) containing 4 ml of simulated intestinal fluid without enzyme (Sigma Aldrich, St. Louis, MO). Samples were incubated in a SteadyShake™ 757 (Amerex Instruments Inc., Concord, CA) incubator at 37 °C and 175 rpm. At 15 min, 30 min, 1 h, 2 h, 3 h, 12 h, and 24 h, 200 μl of release media was removed and replaced. 10 μl aliquots of the release media were combined with 190 μl of 0.025 mg/ml Azure A in a 96-well plate. The absorbance at 620 nm was measured on a Spectramax M2 spectrophotometer (Molecular

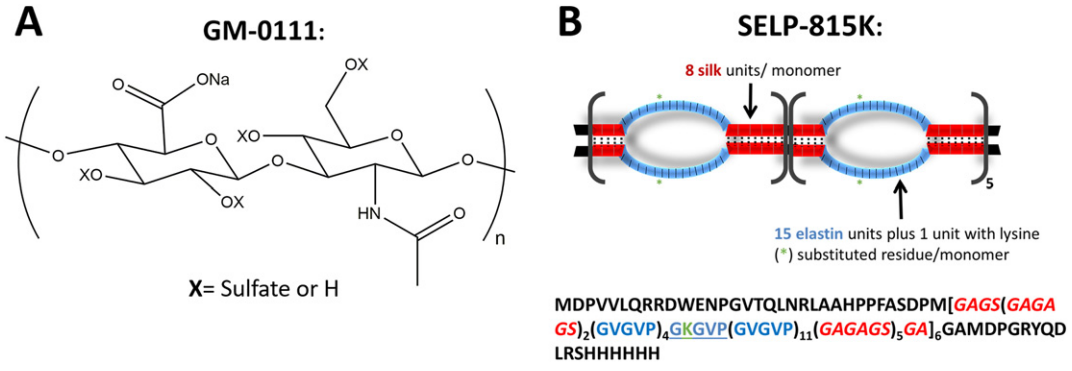

Fig. 1. Structural depiction of: A) GM-0111, and B) SELP 815K with its single letter amino acid sequence.