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New mesalamine polymeric conjugate for controlled release: Preparation, characterization and biodistribution study

Aina L.A. Cesar[a], Fernanda A. Abrantes[d], Luana Farah[a], Rachel O. Castilho[a], Valbert Cardoso[b], Simone O. Fernandes[b], Ivana D. Araújo[c], André A.G. Faraco[a,d,*]

[a] Department of Pharmaceutical Products, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[b] Department of Clinical Analysis, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[c] Department of Surgery, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[d] Department of Aliments, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

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ABSTRACT

Mesalamine (5-ASA) consists of the first-line therapy for the treatment of ulcerative colitis; however, it has low bioavailability, can cause several systemic adverse events, and has low treatment adherence due to the inconvenient dosing scheme. In this work, a new drug delivery system consisting of chondroitin sulfate linked to 5-ASA was synthesized using a carbodiimide as conjugating agent. The system was characterized by spectroscopic techniques (UV, ATR-FTIR, XRD, and NMR ^{1}H) and thermal analysis (TG/DTG and DSC), suggesting the conjugation between the drug and the polymer. The *in vitro* release and the corresponding kinetics were also evaluated, revealing that approximately 40% of the drug linked was released at pH 9 for up to 50 h, following Higuchi's model. The conjugate did not show cytotoxicity for the human monocytic cell line at the doses tested, and an *in vivo* biodistribution study showed that the conjugate remained in the lower GIT for up to 8 h with no uptake in the upper GIT. These data corroborate with the radiation found per segment of GIT and in blood. For this last test the conjugate was radiolabeled with Technetium-99m to allow the scintigraphy evaluation and radiation quantification. In conclusion, the polymeric conjugate was successfully synthesized and demonstrated a mucoadhesiveness on the colon as desired, thus supporting its potential use in the treatment of ulcerative colitis.

1. Introduction

Ulcerative colitis (UC) is a chronic inflammatory disease that affects the colon and rectum, commonly occurring in adolescents and young adults, ages 15 to 30 years old (Ordás et al., 2012; Corridoni et al., 2014). Therefore, the development of new therapeutic alternatives is important since many studies have been reporting an increase in the number of cases in several countries, especially those in the northern hemisphere (Ham and Moss, 2012; da Silva et al., 2014). Its clinical symptoms (diarrhea, abdominal pain, and rectal bleeding) are characterized by periods of exacerbation and remission. As no curative treatments are available, the most promising protocols rely on a long-lasting remission that prevents the relapses (Baumgart and Sandborn, 2007; Canevari et al., 2009; Cañas et al., 2010).

The first-line therapy for mild-to-moderate UC consists of oral administration of mesalamine (5-ASA), since it has demonstrated efficacy in inducing and maintaining remission of the symptoms (Ham and Moss, 2012; Abinusawa and Tenjarla, 2015). Its mechanism of action is still uncertain, but some studies indicate that 5-ASA possibly acts topically on the intestinal mucosa. However, when administered orally, it suffers rapid and extensive absorption on the upper gastrointestinal tract resulting in low bioavailability. For this reason, the administration of high daily doses of 5-ASA is required, which leads to some systemic adverse effects such as blood dyscrasias, pancreatitis, pleuropericarditis, and interstitial nephritis (Mladenovska et al., 2007a; Quetglas et al., 2015).

In order to solve these problems, many approaches have been proposed to increase the 5-ASA bioavailability. An alternative already commercially available is the azo prodrugs, which consist of the 5-ASA molecule bound to an inactive moiety or to another 5-ASA unity *via* azo bonds, for example, Olsalazine and Balsalazide (Quetglas et al., 2015). Bansal et al. (2015) also proposed the conjugation between small molecules. In his work he describes the conjugate benzimidazole-ibuprofen/mesalamine, which presents anti-inflammatory and

* Corresponding author at: Federal University of Minas Gerais, Department of Pharmaceutical Products, Av.Antônio Carlos, 6627 – Pampulha, Belo Horizonte, MG CEP 31270-901, Brazil.
E-mail address: andrefaraco@ufmg.br (A.A.G. Faraco).

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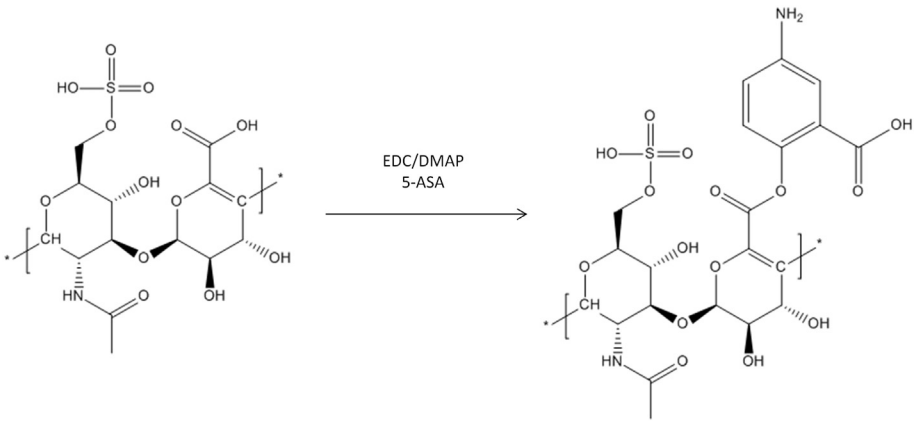

Scheme 1. Description of the synthesis of CS-5-ASA polymer prodrug conjugate.

immunomodulatory activity (Bansal et al., 2015).

In addition, controlled release systems have been developed to increase the bioavailability of the drug through, for example, specific delivery at the site of action. Several approaches have been utilized to deliver drugs specifically to the colon, one of which comprises the polymeric prodrugs also known as drug-polymer conjugates (Duncan, 2003; Kopeček, 2013; Pasut and Veronese, 2007). They are extremely interesting from the pharmacotechnical point of view because they combine the physical and chemical properties of the drug and the polymer used in conjunction.

In the literature we can find some examples of polymer conjugates of 5-ASA with dextrans (Ahmad et al., 2006; Shrivastava et al., 2013) and poly(ethylene glycol) of different molecular weights and structures (Canevari et al., 2009). In the first case, the polymer proposed by Ahmad et al. and Shirastava et al. presented problems of prodrug integrity (stability of the binding between dextran and 5-ASA). It was difficult for the compound to reach the colon; therefore, it was proposed for use in diseases of the proximal colon. Shirastava reports that for the dextran-5-ASA conjugate release, 50% of the drug was released in 25 h in the *in vitro* test placed with intestinal enzymes (Shrivastava et al., 2013). For the polyethylene glycol-5-ASA release, approximately 90% of the drug was released in 250 h; this prodrug has a disadvantage because the polymer has no mucoadhesive properties and may lead to a decrease in the release time of drug action (Varum et al., 2008; Canevari et al., 2009).

On the other hand, chondroitin sulfate is a sulfated glycosaminoglycan and has been commonly used as a dietary supplement for osteoarthritis. Among the works previously reported, and to the best of our knowledge, there is no synthesis of polymeric prodrugs between 5-ASA and chondroitin sulfate reported in the literature. Chondroitin sulfate (CS) is a natural mucopolysaccharide that can be considered an alternative to the production of a prodrug because it has functional groups that can be handled; it is biodegradable, biocompatible, and mucoadhesive (Li et al., 2015); and it is approved by the FDA and widely used in therapeutics for orthopedic treatments (Henrotin et al., 2010).

The mucoadhesiveness capacity could prevent absorption of 5-ASA on the upper gastrointestinal tract, allowing the reduction of the dose required to obtain therapeutic effect. Consequently, this leads to the reduction of adverse effects, suggesting an increase in treatment adherence (Li et al., 2015; Xu et al., 2014).

In this work, we report the development and characterization of the polymeric prodrug obtained through the linkage of CS with 5-ASA, corresponding to a novel molecule and a potential alternative to the treatments currently available for the UC. The system was characterized by spectroscopic technique and thermal analysis. The pH profiles of the conjugate hydrolysis are also reported. *In vitro* assay was performed to evaluate the potential of inhibiting the production of inflammatory cytokine TNF-α. The conjugate was also labeled with technetium-99m in order to evaluate the *in vivo* biodistribution profile and mucoadhesion potential.

2. Materials and methods

2.1. Materials

All chemicals were reagent or analytical grade. Chondroitin sulfate (chondroitin 4-sulfate sodium salt) and 5-ASA were obtained from Galena Chemical and Pharmaceutical Ltda. (Campinas, Brazil). The chondroitin sulfate provided was equivalent to that commercially available; therefore, it consisted of a mixture of isomers of chondroitin 4-sulfate sodium salt, mass average 45,000 Da. Dimethylsulfoxide (DMSO) was supplied by Impex (Novo Hamburgo, Rio Grande do Sul), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and dimethylaminopyridine (DMAP) were supplied by Sigma-Aldrich (Iceland). MTT reagent and DuoSet® ELISA Development Kit were provided by R & D Systems (Minneapolis, USA). Technetium-99m was obtained from an alumina-based $^{99}Mo/^{99m}Tc$ generator.

The human monocytic cell line (THP-1/ATCC TIB-202) was grown in RPMI-1640 medium (Sigma, USA) supplemented with 10% FBS, 100 U/mL of penicillin, and 100 µg/mL of gentamicin at 37 °C, in an atmosphere containing 5% CO_2. The medium was renovated twice a week, when cell concentrations reached 1.0×10^6 cells/mL.

2.2. Methods

2.2.1. Synthesis

The conjugate (CS-5-ASA) was synthesized according to Scheme 1 by a method adapted from Murakami (Murakami and Aoki, 2006). Chondroitin sulfate (2.0 g) was solubilized in anhydrous DMSO, and after complete solubilization, 2 mmol of EDC was added to the solution. Next, 1 mmol and 0.4 mmol of 5-ASA and DMAP were added respectively. The solution was then stirred for 24 h. After completion of the reaction, an excess of acetone was added and the conjugate was isolated by precipitation. Then, the product was put into a membrane bag (14,000 Da) and dialysis was carried out in milliQ water for about 24 h. Finally, the conjugate was lyophilized. The content of 5-ASA linked to CS was determined using spectrometry in the UV region by spectrometry method (Canevari et al., 2009): Accurately weighed CS-5-ASA conjugate (10 mg) was put into a dialysis membrane bag and suspended in 20 mL of 0.1 M borate buffer at pH 9; the content of 5-ASA was read until hydrolysis was completed.

2.2.2. Physicochemical evaluation of CS-5-ASA

The samples 5-ASA, CS, CS-5-ASA, and physical mixture (which is a simple mixture of 5-ASA and CS in the same proportion of reaction - without the occurrence of the reaction) were evaluated by various techniques. ^1H NMR spectra were obtained on a Bruker Avance DPX-