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Research paper

Comparison of mucoadhesive and cohesive features of poly(acrylic acid)-conjugates respective their molecular mass

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ABSTRACT

Objectives: This study aimed to assess the impact of molecular mass as well as the differences between poly(acrylic acid)-thiol-conjugates (PAA100,250,450 KDa) on their mucoadhesive and cohesive qualities.
Methods: Covalent attachment of cysteine (CYS), cysteamine (CYSM) and L-gluthathione (GSH) to poly (acrylic acid) was achieved by formation of amide bonds between primary amino group of the amino acid (in the case of cysteine and glutathione), respectively the amino group of the aminothiol cysteamine and carboxylic acid group of the polymer. Obtained polymer conjugates were evaluated in regard to their safety profile, mucoadhesive properties on the buccal mucosa by rotating cylinder, tensile strength and rheological investigations, respectively. Furthermore, stability, cohesive and water uptake studies were performed.
Key findings: Mucoadhesive studies revealed that maximum detachment force of PAACYS450 was 24.3-fold higher in comparison to the respective controls. Stability studies revealed for PAACYS450 a 50.2-fold higher stability compared to controls.
Conclusion: Taken together, among all polymers tested, PAACYS450 evinced the most favorable qualities regarding mucoadhesion and cohesion, followed by PAACYSM450 and PAACYS250.

1. Introduction

A current key ambition in pharmaceutical technology is finding alternative routes to the parental one. By this approach, it is aimed to make drugs systemically available, without pain, fear and risks. Amidst the divergent routes of drug delivery, oral pathway is one of the most pleasant one for patients [1]. Nevertheless, being orally administrated several detriments such as first pass metabolism and enzymatic degradation inside the gastrointestinal tract, on the basis of this disadvantages oral administration of several classes of drugs are impeded. For that reason, buccal mucosa is on a variety of grounds a very auspicious and promising target area [2]. Comprising rich blood supply, the fact of being relatively permeable and robust, as well as a short recovery time after stress or damage renders the oral mucosa distinguished for the systemic as well as the local application of drugs [3]. A crucial pro of buccal drug delivery systems is an emended patients' compliance favoring the ease of administration, but also circumventing first-pass effect and avoiding acid hydrolysis in the gastrointestinal tract [4]. However, buccal delivery systems have to boast specific traits to overcome diffusion barrier, enzymatic barrier and absorption barrier [5,6]. Apart from this, the barrier represented by efflux pumps limiting the bioavailability of different drugs needs to be surmounted. One of the most encouraging attempts of drug carrier-systems for multifunctional buccal delivery are application systems comprising thiolated polymers or so called thiomers [7].

By further functionalization of well-established polymers, such as poly(acrylic acid) via implementation of thiol-bearing ligands into polymeric backbone, mucoadhesive qualities are substantially amended guaranteeing a sustained and also intimate contact among polymeric delivery system and mucosal membrane [8]. Thiomers offer their mucoadhesive potential by building covalent bonds by formation of disulfide bridges between their sulfhydryl residues and cysteine-rich subdomains of the mucus, due to thiol/disulfide exchange reactions or plain oxidation processes. In this manner, mucoadhesion is immensely fostered compared with carriers absent from thiol bearing residues possessing solely non-covalent bonds [9].

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Therefore, this study aimed to appraise the mucoadhesive and cohesive features of poly(acrylic acid)-conjugates respective unmodified poly(acrylic acid) with regard to their molecular mass.

2. Materials and methods

2.1. Materials

L-Cysteine, cysteamine, 5,5′-Dithiobis-(2-nitrobenzoic acid) DTNB, 1-Ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (EDAC), l-glutathione, hydrochloric acid, potassium phosphate, sodium borohydride, di-sodium hydrogen phosphate, poly(acrylic acid) 100 kDa, poly(acrylic acid) 250 kDa, poly(acrylic acid) 450 kDa, tris (hydroxymethyl) aminomethane hydrochloride, Resazurin salt, Minimum Essential Medium (MEM) and Triton®X-100 were obtained from Sigma-Aldrich (Vienna, Austria). All other reagents used were of analytical grade. Cell culture supplements were purchased from Biochrom AG, Berlin, Germany. Multiwell plates and tissue culture flasks were received from Greiner bio-one, Kremsmünster, Austria. Caco-2 cells were purchased from the European Collection of Cell Culture (ECACC), Salisbury, England.

2.2. Methods

2.2.1. Synthesis of modified Poly(acrylic acid) conjugates

The covalent attachment of cysteine, cysteamine and l-glutathione to poly(acrylic acid) was achieved by the formation of amide bonds between the primary amino group of the amino acid (in the case of cysteine and glutathione), respectively the amino group of the aminothiol cysteamine and the carboxylic acid group of the polymer [10–12]. Briefly, polymer was hydrated in demineralized water and the pH was adjusted to pH 6 by addition of 5 M NaOH. EDAC was added for the purpose of activate the carboxylic acid moieties of the hydrated poly(acrylic acid). After 20 min incubation at room temperature, thiol-constituents cysteine, cysteamine and l-glutathione were added as shown in Table 1 and pH was readjusted to pH 6. Afterwards, resulting conjugates were isolated by dialysing at 10 °C in the dark against 1 mM HCl and 1 mM HCl containing 1% NaCl. Control polymers were prepared in the same way but omitting carbodiimide during the coupling reaction. After dialysis, pH of the solutions were readjusted and mixtures were lyophilized at −30 °C and 0.01 mbar (Christ Gamma 1-16 LSC Freeze dryer) [13].

Afterwards, 30 mg of the lyophilized polymer conjugates were compressed into flat-faced discs. To obtain discs with a diameter of 5.0 mm a single-punch eccentric press (Paul Weber, Germany) was used keeping constant compaction pressure of 11 kN during. The lyophilized polymers were stored at 8 °C until further use [9].

2.2.2. Characterization of obtained conjugates

The amount of thiol groups immobilized on the poly(acrylic acid) backbone were determined photometrically applying Ellman's reagent as described previously by the research group of Bernkop-Schnürch et al. [14]

First of all 0.5–1 mg of samples and controls was dissolved in 500 μL Ellman's buffer consisting of 0.5 M phosphate buffer pH 8 and subsequently 500 μL fresh prepared Ellman's reagent (consisting of 3 mg DTNB dissolved in 1 mL Ellman's buffer) was added to the mixtures incubating at room temperature for 90 min in the dark [15].

A blank-test and a serial dilution were prepared equal to the samples and controls.

Upon completion of incubation aliquots of 100 μL of each solutions were transferred 96 microtiter wellplate and measured at 450 nm using Tecan infinite M200 (Grödig, Austria). Furthermore, disulfide bond test was performed to quantify disulfide bonds formed due to oxidation during the reaction. Determination of disulfide content occurred in a resembling way to thiol group content determination. Initially, samples were dissolved in Tris-buffer (0788 g Tris-HCl in 100 mL demineralized water, adjusted to pH 7.6) and reduced with $NaBH_4$-solution (400 mg $NaBH_4$ solved in 10 mL H_2O). After 2 h of incubation at 37 °C in a shaking bath the reaction was stopped with 250 μL 5 N HCl. Afterwards 1 mL phosphate buffer pH 8 and 100 μL freshly prepared Elman's reagent was added and after 90 min incubating at room temperature in the dark the measurement was accomplished at 450 nm using Tecan infinite M200 (Grödig, Austria).

2.2.3. Cell viability study according to Resazurin assay

Cell viability studies were carried by Resazurin assay. To assess the toxicity of both thiolated and non- thiolated polymers on Caco-2 cells, 1x 10^5 Caco-2 cells were seeded per well in 24-well plates and consecutively incubated in a moisturized chamber at a retained temperature of 37 °C, and 5% CO_2. At a concentration of 0.5% (w/V) the cytotoxicity of tested thiomers as well as controls was examined. As a control conduced minimum essential medium (MEM, pH 7.1–7.4) without phenol red. After an incubation time of 24 h, testing samples were removed and 0.5 mL of Resazurin solution was added. Cells were incubated for further 3 h and subsequently fluorescence of the supernatants was measured at 540 nm excitation and 590 nm emission using Tecan infinite M200 (Grödig, Austria) and cell viability was calculated according following equation [16].

$$\text{Cell viability } (\%) = (\text{Average fluorescence value of each sample} / \text{Average fluorescence of low control}) * 100$$

2.2.4. Evaluation of the swelling behavior

The water-absorbing capacity was determined by a gravimetric method. Test discs of all examined polymers, thiolated as well as non-thiolated were fixed on a needle and submersed in a test tube comprising simulated saliva fluid (2.38 g Na_2HPO_4, 0.19 g KH_2PO_4 and 8.0 g NaCl dissolved in 1 L demineralized water adjusted to pH 6.75 [13]) at 37 °C.

Table 1
Composition of used reagents for reaction mixtures.

Conjugate	Polymer (g/80 mL)	EDAC (mM)	Cysteine (g)	Cysteamine-HCl (g)	Glutathione (g)
PAA_{100}-Cysteine PAACYS100	1.0	200	1.0		
PAA_{250}-Cysteine PAACYS250	1.0	200	1.0		
PAA_{450}-Cysteine PAACYS450	1.0	200	1.0		
PAA_{100}-Cysteamine PAACYSM100	0.8	100		0.75	
PAA_{250}-Cysteamine PAACYSM250	0.8	100		0.75	
PAA_{450}-Cysteamine PAACYSM450	0.8	100		0.75	
PAA_{100}-Glutathione PAAGSH100	1.0	50			2.0
PAA_{250}-Glutathione PAAGSH250	1.0	50			2.0
PAA_{450}-Glutathione PAAGSH450	1.0	50			2.0