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The effects of the thiolation with thioglycolic acid and L-cysteine on the mucoadhesion properties of the starch-graft-poly(acrylic acid)

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ABSTRACT

The aim of this study is to investigate the effects of the thiolation on the mucoadhesion characteristics of the gelatinized and crosslinked wheat starch-graft-poly(acrylic acid) [$(WS\text{-}g\text{-}PAA)_{gc}$] for potential use in drug delivery *via* vaginal route. Thiolation of $(WS\text{-}g\text{-}PAA)_{gc}$ was first time realized using L-cysteine hydrochloride monohydrate (CyS) and thioglycolic acid (TGA). These conjugates [$(WS\text{-}g\text{-}PAA)_{gcth}$] were characterized using FTIR. The free SH group, mucoadhesion, cytotoxicity characteristics and the mechanism of the thiolation were also evaluated. To obtain fundamental data for possible application such as drug carrier, *in vitro* and *in vivo* progesterone release profiles from the mucoadhesive tablet formulations were also determined. The results showed that, vaginal tablet containing $(WS\text{-}g\text{-}PAA)_{gc}$-TGA, which has not contain free SH groups in its structure, displays higher mucoadhesion than $(WS\text{-}g\text{-}PAA)_{gc}$ and $(WS\text{-}g\text{-}PAA)_{gc}$-CyS. This tablet formulation can also be used as a drug carrier in vaginal applications.

1. Introduction

Various natural and synthetic polymers have been used in vaginal bioadhesive formulations as drug delivery systems (Yoo & Lee, 2006). Especially, polysaccharides such as starch, chitosan, hyaluronic acid, cellulose and its derivatives have been used to develop vaginal drug delivery systems due to their biocompability and hydrophilicity (Grabovac, Guggi, & Bernkop-Schnürch, 2005; Khutoryanskiy, 2011; Valenta, 2005). In our previous research, progesterone containing biodegradable and mucoadhesive vaginal tablets based on gelatinized and crosslinked wheat starch-graft-poly(acrylic acid sodium salt) copolymers $(WS\text{-}g\text{-}PAA)_{gc}$ were prepared and evaluated for the drug carrier in the estrus synchronization of ewes for the first time (Gök et al., 2016). In the estrus synchronization of farm animals needs to imitate the function of corpus luteum for at least 10–12 days of luteal phase. Therefore, vaginal formulation, which has prolonged retention time, is preferred for this aim.

Nowadays, a new generation thiolated polymers (thiomers), which have thiol groups bearing side chains, are one of the important mucoadhesive polymers (Bernkop-Schnürch, 2005b). Drug delivery systems prepared with thiomers have many advantages than the other polymer based systems. They show strongly improved cohesive properties by the formation of inter and/or intrachain disulphide bonds resulting higher mucoadhesiveness. Thiolated polymers, which lead to sustained drug release, are used *via* mucosal routes including vaginal one for delivery of antibiotics and antimycotics as well as progesterone and testosterone hormone replacement therapy (Bernkop-Schnürch, Kast, & Richter, 2001; Bernkop-Schnürch & Steininger, 2000; Cevher, Sensoy, Taha, & Araman, 2008; Greindl & Bernkop-Schnürch, 2006; Kafedjiiski, Krauland, Hoffer, & Bernkop-Schnürch, 2005; Kast & Bernkop-Schnürch, 2001; Trimnell, Stout, Doane, & Russell, 1977; Valenta, Kast, Harich, & Bernkop-Schnürch, 2001).

In this study, the effects of the thiolation reagents on the mucoadhesion and drug carrier characteristics of the $(WS\text{-}g\text{-}PAA)_{gc}$ were investigated. For this purpose, thiolation of $(WS\text{-}g\text{-}PAA)_{gc}$ was first time carried out using two different thiolation reagents CyS and TGA. Characterization of their structures and the determination of the properties of the $(WS\text{-}g\text{-}PAA)_{gcth}$ were realized. Cytotoxicity of

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$(WS\text{-}g\text{-}PAA)_{gcth}$ on HEK 293T cell line were evaluated. Availability of these conjugates as mucoadhesive drug carrier *via* vaginal route was also examined *in vitro* and *in vivo*.

2. Materials and methods

2.1. Materials

$(WS\text{-}g\text{-}PAA)_{gc}$ with a grafting degree (G%) of 23.34 was synthesized according to the conventional chemically initiated (using ammonium cerium-IV-nitrate (CAN)-HNO_3 initiator system) aqueous media free radical addition polymerization in our laboratory (Gök et al., 2016).

Poly(acrylic acid) (PAA) (M_w ~750 kDa), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDAC), CyS and TGA were also purchased from Sigma-Aldrich (USA). Other reagents were chemically pure grade and all solutions were prepared with deionized water.

Progesterone (4-Pregnene-3,20-dione; powder, BioReagent and suitable for cell culture) was purchased from Sigma-Aldrich (USA). pH = 5 lactate buffer solution was used as *in vitro* vaginal medium (Gök et al., 2016). Progesterone Radioimmunoassay kit (Immunotech RIA Progesterone) was purchased from Beckman Coulter Company (France). Other chemicals were of analytical grade.

2.2. Synthesis of $(WS\text{-}g\text{-}PAA)_{gcth}$

Synthesis of $(WS\text{-}g\text{-}PAA)_{gcth}$ were first realized by thiolation of $(WS\text{-}g\text{-}PAA)_{gc}$ which was carried out according to the EDAC-mediated coupling reaction (Bernkop-Schnürch et al., 2001). To protect sulfhydryl groups from the oxidation, the reaction was performed at pH 5 under nitrogen gas stream at room temperature

A slurry mixture of 1 g $(WS\text{-}g\text{-}PAA)_{gc}$ was dispersed in 250 mL deionized water, then EDAC (50 mM) and thiolation reagent (4 g; CyS or TGA) were added and kept at room temperature for 3 h. To purify the $(WS\text{-}g\text{-}PAA)_{gc}$-CyS and $(WS\text{-}g\text{-}PAA)_{gc}$-TGA, the reaction mixture was dialyzed against 5 mM hydrochloric acid at $10 \pm 1\,^{\circ}C$ (dialyze tubes Medicell International Ltd, Size 3 Inf Die 20/32-15.9 mm, MWCO-12-14,000 Da) once, against 5 mM HCl containing 1% sodium chloride twice and against 1 mM HCl containing 1% sodium chloride twice. Following the adjustment of pH of the thiolated copolymer conjugates to 5, it was lyophilized at 0.01 mbar pressure at $-30 \pm 1\,^{\circ}C$ (Lyovac GT2E, Steris, Germany) and the $(WS\text{-}g\text{-}PAA)_{gc}$-CyS and $(WS\text{-}g\text{-}PAA)_{gc}$-TGA were kept at $+4\,^{\circ}C$. For comparison, wheat starch (WS) and PAA were thiolated, having the same experimental conditions. All conjugates were characterized using FTIR spectroscopy (Digilab Excalibur-FTS 3000MX, USA) technique.

2.3. Determination of the free thiol (SH) group of the $(WS\text{-}g\text{-}PAA)_{gcth}$

The amount of free SH groups of the conjugates was determined by iodometric titration (Bernkop-Schnürch & Steininger, 2000). The results were presented as (μmol/g polymer). Each experiment was carried out in triplicates.

2.4. Preparation of the tablet for the swelling and mucoadhesion studies

To evaluate the swelling, erosion and mucoadhesion properties of the conjugates, tablets of 13 mm in diameter disc, containing 70 mg dried powder samples, were prepared by compression on a laboratory tablet press (Korsch EK-0, Germany) at 5,000 psi pressure.

2.4.1. Determination of equilibrium swelling degree (Q_e) and matrix erosion (ME%) of the tablets

The swelling behavior and the erosion characteristics of the tablets were investigated in pH = 5 lactate buffer solution as *in vitro* vaginal medium at $37.0 \pm 0.1\,^{\circ}C$, gravimetrically. Q_e and ME% were determined and calculated according to the tea bag method (Gök et al., 2016).

2.4.2. Mucoadhesion studies

In vitro mucoadhesion of the tablets was evaluated using a TA-XT Plus Texture Analyser (Stable Microsystems, Haslemere, UK) equipped with a 5 kg load cell according to a previously described method (Gök et al., 2016). Freshly excised ewe vaginal mucosa obtained from slaughterhouse was used. Each experiment was carried out in triplicate.

2.5. Preparation of tablet formulations for in vivo studies

To observe the behaviour of the tablet definitely and easily inside the ewe vagina, trypan blue as a nontoxic and inert dyestuff was used the tablet preparation. Magnesium stearate as a lubricant was also added to tablet formulation. 1.5 g $(WS\text{-}g\text{-}PAA)_{gcth}$ which was passed through the 250 mm sieve, 2.4 mg trypan blue and 0.4 mg magnesium stearate were mixed in cubic mixer for 2 min. Powder mixture compressed using the technique explained in Section 2.4. Tablet formulation, which was used pharmacokinetic studies, contains 1.5 g $(WS\text{-}g\text{-}PAA)_{gc}$-TGA, 0.4 mg magnesium stearate and 75 mg progesterone instead of trypan blue. Production batch size of the each tablet formulation containing progesterone was around 100 tablets.

2.6. In vitro drug release studies

To perform *in vitro* drug release studies, rotating basket method at 75 rpm was used (Gök et al., 2016). Mucoadhesive tablet formulations [$(WS\text{-}g\text{-}PAA)_{gc}$-CyS and $(WS\text{-}g\text{-}PAA)_{gc}$-TGA] used *in vitro* assay were compared with $(WS\text{-}g\text{-}PAA)_{gc}$, two commercially available products such as controlled internal drug release device (CIDR) which contains 330 mg the natural hormone progesterone and controlled release intravaginal sponge which was impregnated 20 mg flugestone acetate. A dissolution medium used in the assay contained 500 mL lactate buffer with (for formulations containing water insoluble progesterone) or without (for formulation containing water soluble flugestone acetate) 0.25% sodium lauryl sulphate. *In vitro* assay was carried out at $37.0 \pm 0.5\,^{\circ}C$. An aliquot of sample was withdrawn at predetermined times (from 0 to 72 h) and flugestone acetate was analyzed spectrophotometrically (Shimadzu UV-1601, Japan) at 240 nm. Progesterone was also analyzed by modified HPLC method (Gök et al., 2016).

2.7. In vivo studies

The live weights of the healthy 3–4 aged native Anatolian sheep (Ovis aries) breeds, Red Karaman, Gokceada and Kivircik ewes, which were used *in vivo* assay, were approximately 35–45 kg.

2.7.1. In vivo determination of retention time of mucoadhesive vaginal tablets

In order to generate the proper tablet formulation, the *in vivo* retention time test was primarily applied to evaluate the behaviour of vaginal tablets (appearance, physical changes, swelling behaviours and residence time in vagina *etc.*) containing trypan blue in ewe vagina without using the hormone. Tablets, which were prepared with $(WS\text{-}g\text{-}PAA)_{gc}$-CyS and $(WS\text{-}g\text{-}PAA)_{gc}$-TGA and had an optimal disintegration time (Table 2), were placed into the vaginal apex using tablet applicator. Tablet was applied in 4