Development and *In Vitro* Evaluation of a Mucoadhesive Vaginal Delivery System for Nystatin

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ABSTRACT: The purpose of the present study was to design and evaluate a novel vaginal delivery system for nystatin based on mucoadhesive polymers. L-Cysteine and cysteamine, respectively, were covalently attached to poly(acrylic acid), and the two different thiolated polymers were evaluated *in vitro* regarding their swelling behavior, mucoadhesive properties and release behavior. Tablets comprising these thiolated polymers and nystatin demonstrated a high stability in vaginal fluid simulant pH 4.2 and an increase in weight by swelling whereas control tablets comprising unmodified poly(acrylic acid) disintegrated and dissolved. The mucoadhesion time of tablets on freshly excised bovine vaginal mucosa on a rotating cylinder and the total work of adhesion of gels and tablets increased significantly due to the formation of disulfide bonds between the thiolated polymer and cysteine rich subdomaines of the mucus layer. The drug nystatin was released more slowly out of thiomer tablets and gels than out of PAA control tablets and gels. Therefore these thiolated polymers are promising delivery systems for nystatin providing a prolonged residence time and a sustained drug release *in vitro* under physiological relevant conditions. © 2008 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 98:555–564, 2009

Keywords: polymers; polymer synthesis; gels; controlled release; polymeric drug delivery system; thiomer; oscillatory rheology; mucoadhesion; nystatin; vaginal

INTRODUCTION

Within the last decade the interest in vaginal drug delivery has increased. Different studies show that the vaginal route is an attractive alternative way of application to the oral route for systemic drug delivery especially for proteins and peptides.[1–3] The vaginal route of administration can be used for local application as well as for systemic drug delivery. Advantages are a large surface area, a rich blood supply, no first-pass effect and its good permeability for many drugs.[4] However, changes in the membrane during the menstrual cycle and the vaginal fluid are limiting factors. One problem is that drug delivery systems do not remain sufficiently long in the vaginal cavity because they are washed out by the vaginal fluid quickly. Therefore they have to be applied frequently which reduces the patient compliance.

Many drug delivery systems are based on polymers, which are mucoadhesive, swell rapidly in an aqueous environment and exhibit a controlled drug release. In the last years new polymers could be synthesized by the introduction of thiol groups. These thiolated polymers or so-called thiomers are able to form covalent bonds with cysteine rich sub-domains of mucus

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glycoproteins[5] and show strongly improved mucoadhesive properties.[6] Because of these strong mucoadhesive properties, poly(acrylic acid)–cysteine conjugate and the new poly(acrylic acid)–cysteamine conjugate were chosen as model polymers for this study.

Vaginitis caused by candida albicans is one of the most common gynological diseases which approximately 75% of women experience at some time in their life.[7] For the treatment of vaginitis local antifungal chemotherapy is preferred over systemic administration for toxic reasons. The polyene antifungal nystatin was therefore chosen as a drug because it is frequently used and of high practical relevance in treatment of vaginitis, negligibly absorbed after vaginal application and safe throughout pregnancy.[8]

It is therefore the aim of this study to develop a more effective mucoadhesive vaginal delivery system for nystatin which resides at the vaginal cavity for a prolonged period of time and guarantees a sustained release over this period. The two thiomers were evaluated *in vitro* regarding the amount of covalently attached thiol groups, their swelling behavior, their viscoelastic properties, their mucoadhesive properties and their drug release behavior.

MATERIALS AND METHODS

Materials

Poly(acrylic acid) (average molecular mass: 450 kDa) (PAA), L-cysteine hydrochloride, cysteamine hydrochloride, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC), 5, 5′-dithiobis(nitrobenzoic acid) (Ellman's reagent) and 2,4,6-trinitrobenzenesulfonic acid (TNBS) were obtained from Sigma–Aldrich (Vienna, Austria). Nystatin (NYS) was purchased from Fagron GmbH & Co. KG (Barsbüttel, Germany). All other reagents were of analytical grade and received from commercial sources.

Synthesis of the Poly(Acrylic Acid) Conjugates

The covalent attachment of L-cysteine and cysteamine to poly(acrylic acid) was achieved by the formation of amide bonds between the primary amino group of the amino acid and the carboxylic acid group of the polymer. The poly(acrylic acid) conjugates were synthesized according to a method described previously.[9] Briefly, EDAC was added in a final concentration of 100 mM to a neutralized 1% (w/v) solution of PAA. After activation of the carboxylic acid moieties for 15 min, 1 g of L-cysteine HCl or 0.75 g of cysteamine HCl was added and the reaction mixture was incubated at pH 6 for 3 h at room temperature. Unbound L-cysteine was removed via dialysis in cellulose membrane tubings with a molecular weight cut-off of 12 kDa (Sigma–Aldrich) at 10°C in the dark twice against 0.2 mM HCl, twice against the same medium but containing 1% NaCl and finally two times exhaustively against 0.2 mM HCl. Afterwards the pH of the polymer solutions was adjusted to 4 and frozen at −70°C (Refco, Knoxville, TN). Finally the frozen aqueous polymer solutions were lyophilized (−78°C, 0.01 mbar, VirTis, Gardiner, ME) and stored at 4°C until further use. Controls were prepared in exactly the same way but omitting EDAC during the reaction.

Determination of the Thiol Group Content

The amount of thiol groups immobilized on the PAA backbone was determined photometrically with Ellman's reagent quantifying free thiol groups.[10] First, 0.5 mg of each conjugate and the control were hydrated in 500 μL of 0.5 M phosphate buffer pH 8.0 and then 500 μL of Ellman's reagent (3 mg dissolved in 10 mL of 0.5 M phosphate buffer pH 8.0) was added. After incubation at room temperature protected from light for 2 h and centrifugation with 13400 rpm for 5 min (Minispin, Eppendorf, Vienna, Austria) 200 μL of each sample was transferred into a microplate and the absorption was measured at a wavelength of 450 nm using a microplate reader (FluoStar Galaxy, BMG, Offenburg, Germany). The amount of thiol groups was calculated from a standard curve of L-cysteine hydrochloride and cysteamine hydrochloride, respectively, in the concentration range 10–300 nmol/mL prepared in exactly the same way as the samples. The amount of thiol groups on the polymer was calculated by the difference of the amount of thiol groups and the amount of free L-cysteine and cysteamine, respectively.

Determination of Free L-Cysteine/Cysteamine

The amount of free L-cysteine and cysteamine respectively was determined photometrically with TNBS reagent. TNBS reacts with the primary amino group of L-cysteine and cysteamine in a

nucleophilic aromatic substitution developing an orange dye. First, 0.5 mg of each conjugate and the control were hydrated in 500 μL of 0.5% NaCl and then 500 μL of TNBS solution (200 μL TNBS dissolved in 10 mL of 8% $NaHCO_3$-solution) was added. After incubation at 37°C for 1.5 h and centrifugation with 13400 rpm for 5 min (Minispin) 200 μL of each sample was transferred into a microplate and absorption was measured at a wavelength of 450 nm using a FluoStar microplate reader. The amount of free L-cysteine and cysteamine in the polymers was calculated from a standard curve of L-cysteine hydrochloride and cysteamine hydrochloride, respectively, in the concentration range 5–160 nmol/mL prepared in exactly the same way as the samples.

Particle Size Determination of Nystatin

The particle size distribution was determined using a laser diffraction particles size analyzer (analysette 22 compact version, Fritsch GmbH, Idar Oberstein, Germany). Nystatin was dispersed in low viscous silicone oil WACKER AK 10 (viscosity 10 mPa s ± 10% (25°C), Wacker/Hüls, Nünchritz, Germany). The particle suspension was prepared with an ultrasonic stick (Dr. Hielscher GmbH, ultrasonic processor UP200H) before analysis. In the measurement cell, the use of a propeller mixer (dispersion equipment, Fritsch GmbH) facilitate the continuous flux of particles. For the calculation of the particle size determination the Fraunhofer model was used.

Preparation of Test Discs

For discs without NYS 30 mg of the lyophilized polymers or polymer conjugates were compressed into 5.0 mm diameter flat-faced discs with a constant compaction pressure of 5 kN during the preparation of all discs (Manual tablet press, Paul Weber, Remshalden-Grunbach, Germany).

For discs containing NYS first 20 mg of the lyophilized polymers or polymer conjugates were dissolved in 2 mL of water. Afterwards 10 mg NYS was suspended in the polymer solution and the polymer solution was frozen at −70°C. After lyophilization (at −78°C, 0.01 mbar) discs were compressed as described above.

Preparation of Nystatin Gels

Four hundred milligrams of the conjugates or the control were hydrated in 8 mL water. Then the pH of the gel was adjusted to 5.8 with 2 M NaOH. Afterwards phosphate buffer was added to a final conjugate concentration of 4% (w/v) and 200 mg of nystatin was suspended in the gel. The gel was filled into aluminum tubes (Aponorm, WEPA, Hillscheid, Germany) to protect the gel from evaporation.

Evaluation of the Swelling Behavior

The water absorbing capacity was determined by a gravimetric method as described previously by our research group.[11] Test discs of lyophilized polymer–cysteine conjugate, polymer–cysteamine conjugate and controls with and without NYS, respectively, were prepared as described above. Test discs were fixed on a needle and incubated in vaginal fluid simulant (VFS)[12] pH 4.2 at 37°C. At predetermined time intervals the swollen test discs on the needle were taken out of the incubation medium and the amount of water uptake was determined gravimetrically after removal of unbound water on the surface.

Oscillatory Rheology

The viscoelastic properties of the gels of the PAA conjugates were determined with a HAAKE MARS (Haake GmbH, Karlsruhe, Germany), a thermostatically controlled plate-plate rheometer with a plate 35 mm in diameter. All rheological measurements were shear stress controlled whereas the shear stress increased from 0 to 500 Pa. Gels containing NYS were prepared as described above and incubated at 37°C. After determination of the linear viscoelasticity range dynamic oscillatory tests were performed for aliquots of about 750 μL of gels at a frequency of 1 Hz at predetermined time points over a time period of 24 h. Throughout the experimental period, the plate temperature was maintained at 37.0 ± 0.1°C and the gap was 0.5 mm. The parameters obtained thereby were the phase angle (δ) and the complex modulus (G^*). The elastic modulus (G'), the viscous modulus (G'') and the dynamic viscosity (η^*) were calculated by

$$G' = G^* \cos\delta, \qquad G'' = G^* \sin\delta, \qquad \eta^* = \frac{G''}{2\pi\nu}$$

where ν is the oscillatory frequency. Loss tangent ($\tan\delta$), a parameter that represents the ratio between the viscous and the elastic properties of the polymer was also calculated ($\tan\delta = G''/G'$).

Decrease of Thiol Groups

In parallel to the viscosity studies, the degree of disulfide bond formation was monitored. At the same time points when the viscosity was measured, aliquots of 50 mg of each gel were withdrawn. To quench any further reaction, 50 μL of 1 M HCl was added and the samples were stored at −20°C. Afterwards, the decrease of free thiol groups was determined using Ellman's reagent as described above.

In Vitro Evaluation of the Mucoadhesive Properties

Tensile Studies

Thirty milligrams of the conjugates and the control with and without NYS, respectively, were pressed to flat-faced discs as described above. Following this, tensiometer studies with these test discs were carried out on native bovine vaginal mucosa as described by our research group previously.[6] Test discs were attached to the mucosa with a force of 3 mN. After a contact time of 30 min in VFS pH 4.2 the mucosa was pulled at a rate of 0.1 mm/s from the disc. The total work of adhesion (TWA) representing the area under the force/distance curve and the maximum detachment force (MDF) were determined using the WINWEDGE software (TAL Technologies, Inc., Philadelphia, PA) in combination with EXCEL 2003 (Microsoft).

Gels were prepared as described above and tensiometer studies were carried out in a slightly modified way. Two grams of the gels were put into a small beaker and mucosa was attached to the gel with a force of 5 mN. After a contact time of 20 min, the beaker with the gel was pulled at a rate of 0.1 mm/s from the mucosa. The TWA and MDF were then calculated as described above.

Mucoadhesion Studies at the Rotating Cylinder

As established by our research group earlier[13] discs prepared as described above were attached to freshly excised bovine vaginal mucosa, which had been glued with a cyanoacrylate adhesive (Loctite, Henkel, Austria) to a stainless steel cylinder (diameter: 4.4 cm; height: 5.1 cm; apparatus four-cylinder, USP XXIV). Thereafter, the cylinder was placed in the dissolution apparatus according to the USP containing VFS pH 4.2 at 37 ± 1°C. The fully immersed cylinder was agitated with 125 rpm. The detachment time of tablets was evaluated visually.

Release Studies

Release studies of NYS were performed in the paddle dissolution apparatus according to the Ph. Eur. Tablets containing 10 mg of NYS and 20 mg of PAA, PAA–cysteine, or PAA–cysteamine, respectively, were prepared and placed in vessels containing 500 mL VFS pH 4.2 at 37 ± 1°C. Additionally gels containing 2% (w/v) NYS and 4% (w/v) PAA, PAA–cysteine, or PAA–cysteamine were prepared as described above and 1 g of each gel was placed in a vessel containing 900 mL VFS pH 4.2 at 37 ± 1°C. The paddles were agitated with 20 rpm. Sink conditions were maintained throughout the whole study. Samples of 1 mL were withdrawn at predetermined time points over a time period of 1 week and withdrawn samples were replaced with an equal volume of release medium. Released NYS was assayed by measuring the absorbance photometrically at 305 nm. Concentrations were calculated by interpolation from a standard curve with increasing amounts of NYS and PAA.

Statistical Data Analysis

Each experiment was performed in triplicate. Statistical data analysis were performed using Student's *t*-test with $p < 0.05$ as the minimal level of significance.

RESULTS AND DISCUSSION

Characterization of the Synthesized Thiomers

L-Cysteine and cysteamine were attached covalently to poly(acylic acid) under the formation of amide bonds (Fig. 1). The carboxylic acid moieties of the polymer were activated by EDAC forming an *O*-acylurea derivate as intermediate product, which reacts with the primary amino group of L-cysteine or cysteamine. The purification by dialysis was successful as only 6 ± 2 μmol/g polymer thiol groups and 4 ± 1 μmol/g polymer amino groups (Tab. 1) representing the free

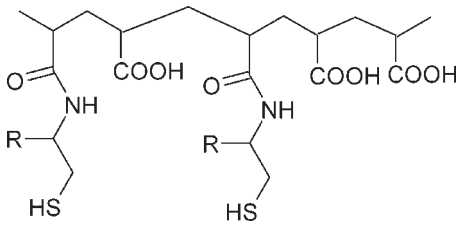

Figure 1. Chemical substructure of used thiolated polymers: PAA–cysteine (R = COOH) and PAA–cysteamine (R = H).

unbound remaining L-cysteine or cysteamine could be detected in control samples.

The lyophilized polymer–cysteine and polymer–cysteamine conjugates appeared as white, odorless powder of fibrous structure and were easy hydratable in aqueous solutions. The amount of free thiol groups were quantified via Ellman's reagent and the amount of remaining amino groups via TNBS reagent. The results are shown in Table 1. PAA–cysteine shows 356 ± 40 μmol/g thiomer thiol groups bound to polymer and PAA–cysteamine 349 ± 42 μmol/g thiomer. Therefore both thiomers exhibit almost the same amount of thiol groups attached, whereas the amount of free cysteamine is 2.5-fold higher than the amount of free L-cysteine. A possible explanation for this fact is that the electron density at the N of cysteamine is higher than in cysteine because in the cysteine molecule the carboxyl group is pulling electrons. The C in the PAA is also poor in electrons and can therefore electrostaticly bind the electron rich N of cysteamine but not the cystein. Cysteamine is more cationic and it is therefore more difficult to separate it from an anionic polymer.

Particle Size Determination of Nystatin

Because of the poor solubility of nystatin in water it is not dissolved but suspended in the prepared gels. Therefore it is of interest how big these particles really are. The smaller the particles are, the greater is their surface which has an influence on the dissolution for example.

Furthermore for the rheology the gap should be at least 10 times bigger than particles in the fluid which is measured. To ensure this and to determine an appropriate gap thickness, the size of the nystatin particles was measured.

The size distribution of NYS particles is shown in Figure 2. The mean is 9.5 ± 3.1 μm with a monomodal distribution.

Evaluation of the Swelling Behavior

The swelling behavior of mucoadhesive polymers has a great influence on their adhesive properties and cohesiveness.[14] Mucoadhesive polymers take water from the underlying tissue by absorbing, swelling and capillary effects which leads to a considerably strong adhesion.[15] Water uptake studies were carried out with tablets of PAA control, PAA–cysteine, and PAA–cysteamine with and without NYS and demonstrated that the covalent attachment of thiol groups has a significant influence on the swelling behavior of the polymers. Thereby obtained results are shown in Figure 3. All polymer tablets except the control both with and without NYS demonstrated a high stability and cohesiveness during the hydration process in aqueous medium as inspected visually. The water uptake of the PAA–cysteamine tablets was less (15-fold) than the one of the PAA–cysteine tablets (18-fold) after 2 h. This results from the fact that the bound cysteine has in contrast to the bound cysteamine a carboxyl group. This additional carboxyl group is able to form hydrogen bonds with the water molecules. Therefore more water than in the cysteamine conjugate tablets can be absorbed.

Table 1. Characterization of the Polymers

	Free L-Cysteine/Cysteamine (μmol/g thiomer ± SD)	Thiol Groups on the Polymer (μmol/g thiomer ± SD)	Acid Groups of PAA Substituted by Thiol Groups (%)
PAA–cysteine	21 ± 3	377 ± 37	2.66 ± 0.30^b
PAA–cysteamine	50 ± 4^a	399 ± 38	2.57 ± 0.31^c
PAA control	4 ± 1	6 ± 2	0.01 ± 0.01

Amount of covalently attached thiol groups per gram thiomer, free L-cysteine/cysteamine and acid groups of PAA substituted by thiol groups. Indicated values are means of at least three determinations.

[a] Differs from PAA–cysteine ($p < 0.005$).

[b] Differs from PAA control ($p < 0.0001$), does not differ from PAA–cysteamine ($p > 0.4$).

[c] Differs from PAA control ($p < 0.0001$).

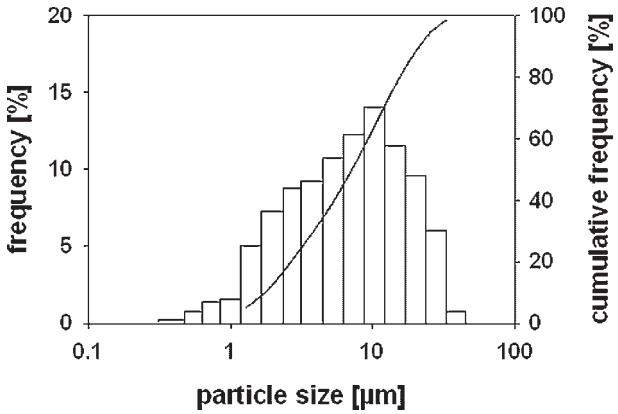

Figure 2. Cumulative frequency and histogram of frequency distribution of NYS.

The weight of the PAA–cysteine/NYS tablets and PAA–cysteamine/NYS tablets increased only seven- and ninefold, respectively, which is not significantly different. Nystatin is hydrophobic and poorly water soluble. Therefore the tablets with nystatin swell slower and do not take as much water as tablets without nystatin.

In contrast the control discs absorbed only in the first 20 min a little water and dissolved then. The swelling behavior of PAA control tablets and PAA–cysteine tablets in a medium with pH 4.2 is the same compared to pH 6.8 which was done by Guggi et al.[16]

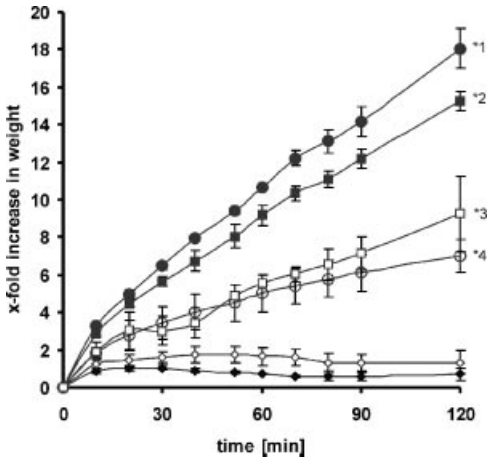

Figure 3. Comparison of the water uptake properties of tablets (30 mg) based on unmodified PAA (◆), PAA–cysteamine (■), PAA–cysteine (●), unmodified PAA/NYS 2:1 (◇), PAA–cysteamine/NYS 2:1 (□) and PAA–cysteine/NYS 2:1 (○) in VFS pH 4.2 at 37 ± 1°C. Indicated values are means (±SD) of three experiments. *1 differs from PAA control ($p < 0.001$). *2 differs from PAA control ($p < 0.002$), differs from PAA–cysteine ($p < 0.05$). *3 differs from PAA control + NYS ($p < 0.01$). *4 differs from PAA control + NYS ($p < 0.01$), does not differ from PAA–cysteine + NYS ($p > 0.1$).

Correlation Between Oscillatory Rheology and Decrease of Thiol Groups

Throughout the evaluation process the modified and unmodified polymer gels exhibited a pseudoplastic behavior (data not shown) which means a decrease in viscosity at an increasing shear rate. Due to the knowledge that thiolated polymers are viscoelastic systems[17] the measurement was done in an oscillatory mode which means a sinusoidal motion back and forward of the plate. The elastic modulus (G'), the viscous modulus (G'') and the dynamic viscosity (η^*) were calculated by the measured parameters phase angle (δ) and the complex modulus (G^*) as described above.

The dynamic viscosity of the PAA gel containing 2% (w/v) of NYS decreased about 30% within 24 h. The more nystatin was in the gel, the more pronounced the decrease was (data not shown). It can be assumed that NYS fluidizes the gel. The viscous modulus (G'') was always greater than the elastic modulus (G'). As $\tan\delta$ is the ratio G''/G' a small value of $\tan\delta$ means a dominating G'. The smaller $\tan\delta$ the higher the elasticity is. $\tan\delta$ of the PAA gel is greater than 1 during the whole examination time, which indicates that it is more a liquid than a gel. In contrast, the dynamic viscosity, the viscous modulus (G'') and the elastic modulus of the PAA–cysteine gel were constant during the examination time of 24 h as shown in Figures 4A and 5A. The amount of thiol groups decreased to 55% of the initial concentration. This can be explained by the formation of inter- and/or intramolecular disulfide bonds within the thiomer. The viscosity did not increase as it would be assumed. Marschutz et al. showed the increase of viscosity because of disulfide bond formation in a PAA–cysteine gel 3% (m/v) but without a drug.[9] The PAA–cysteamine gel became more and more liquid within 24 h. Although the amount of thiol groups decreased to 60% of the initial value (Fig. 5B), the viscosity, the elastic modulus and the viscous modulus decreased rapidly (Figs. 4B and 5B). It has been shown earlier, that free L-cysteine decreases the mucoadhesion and the viscosity of the polymer.[5] The existence of relatively more free cysteamine in the PAA–cysteamine gel than free L-cysteine in the PAA–cysteine gel can explain the decrease in viscosity.

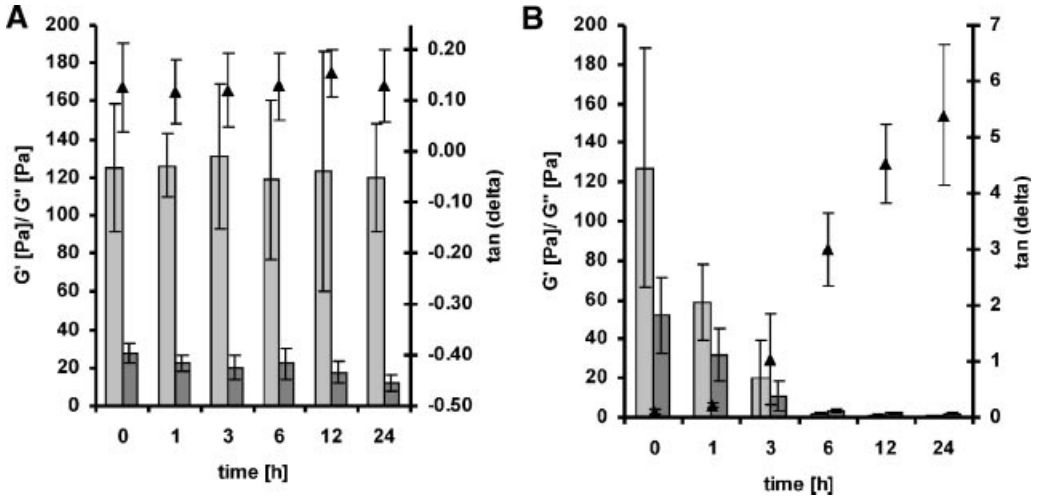

Figure 4. (A) Comparison of elastic modulus G' (light gray bars), viscous modulus G'' (dark gray bars) and loss tan (▲) of PAA–cysteine 4% (w/v). Oscillatory measurements were carried out at 1 Hz frequency at 37°C. All indicated values are means (±SD) of three experiments. (B) Comparison of elastic modulus G' (light gray bars), viscous modulus G'' (dark gray bars) and loss tan (▲) of PAA–cysteamine 4% (w/v). Oscillatory measurements were carried out at 1 Hz frequency at 37°C. All indicated values are means (±SD) of three experiments.

In Vitro Evaluation of the Mucoadhesive Properties

Tensile Studies

The results of the tensile studies of the discs and the gels are shown in Figure 6A and B, respectively. The TWA of the tablets of PAA–cysteine/NYS and PAA–cysteamine/NYS were 2.1- and 1.9-fold higher than the PAA control tablets. Also the MDF was increased 2.3- and 1.9-fold, respectively. There is no significant difference between the two different thiomers ($p > 0.3$ and $p > 0.3$, respectively).

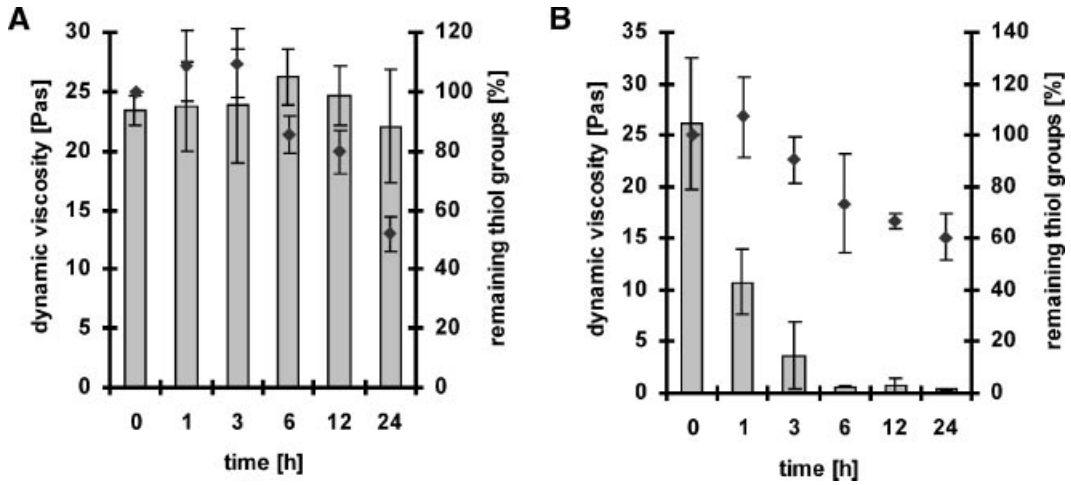

Figure 5. (A) Correlation between dynamic viscosity (gray bars) and remaining thiol groups (◆) quantified via Ellman's reagent of PAA–cysteine 4% (w/v). Oscillatory measurements were carried out at 1 Hz frequency at 37°C. All indicated values are means (±SD) of three experiments. (B) Correlation between dynamic viscosity (gray bars) and remaining thiol groups (◆) quantified via Ellman's reagent of PAA–cysteamine 4% (w/v). Oscillatory measurements were carried out at 1 Hz frequency at 37°C. All indicated values are means (±SD) of three experiments.

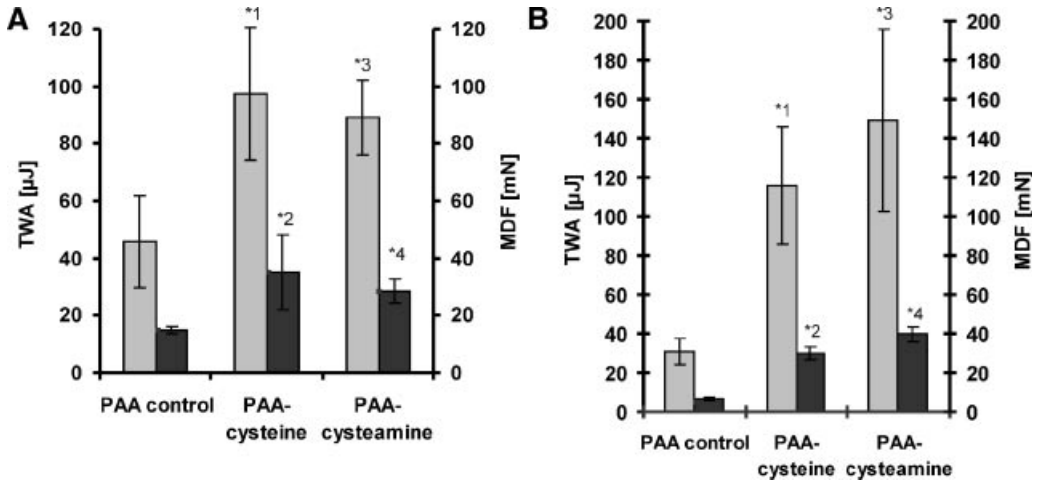

Figure 6. (A) Comparison of the TWA (gray bars) and MDF (black bars) of tablets containing 10 mg of NYS and 20 mg of unmodified PAA, PAA–cysteine and PAA–cysteamine, respectively, in VFS pH 4.2 at bovine vaginal mucosa. Indicated values are the means (±SD) of at least three experiments. *1 differs from PAA control ($p < 0.05$). *2 differs from PAA control ($p < 0.05$). *3 differs from PAA control ($p < 0.01$), does not differ from PAA–cysteine ($p > 0.3$). *4 differs from PAA control ($p < 0.01$), does not differ from PAA–cysteine ($p > 0.35$). (B) Comparison of the TWA (gray bars) and MDF (black bars) of gels containing 2% (w/v) nystatin and 4% (w/v) unmodified PAA, PAA–cysteine, and PAA–cysteamine, respectively, with bovine vaginal mucosa. Indicated values are the means (±SD) of at least three experiments. *1 differs from PAA control ($p < 0.02$). *2 differs from PAA control ($p < 0.01$). *3 differs from PAA control ($p < 0.02$), does not differ from PAA–cysteine ($p > 0.1$). *4 differs from PAA control ($p < 0.01$), does not differ from PAA–cysteine ($p > 0.05$).

The TWA of the gel of PAA–cysteine containing nystatin was 3.8-fold higher as the TWA of the PAA/nystatin control gel, and the MDF was even 4.5-fold higher. The PAA–cysteamine gel containing nystatin showed a 4.9-fold increased TWA and a 6-fold increased MDF which is not significantly different from the PAA–cysteine/NYS gel ($p > 0.1$ for TWA and $p > 0.05$ for MDF).

Mucoadhesion Studies at the Rotating Cylinder

The test system on the rotating cylinder is assumed to be closer to the *in vivo* situation than simple tensile studies described above, because the cohesiveness of the polymers is also taken into account. The results of mucoadhesion studies performed with the rotating cylinder method are shown in Figure 7. The adhesion time of the PAA–cysteine tablets was 3.5-fold higher and the adhesion time of PAA–cysteine/NYS tablets was 1.7-fold greater than the adhesion time of the PAA control without and with NYS. In addition the adhesion time of the PAA–cysteamine and the PAA–cysteamine/NYS tablets increased 4.9- and 1.7-fold, respectively, compared with the control.

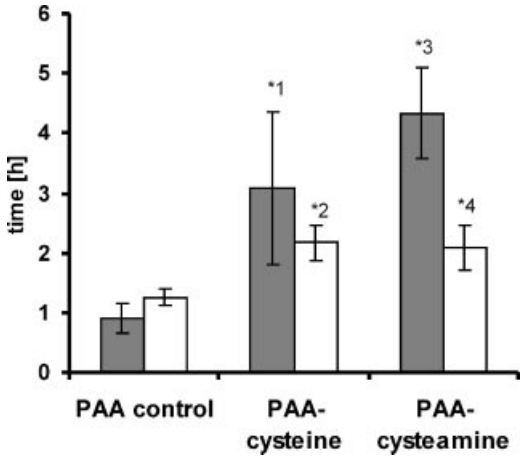

Figure 7. Comparison of the adhesion time of thiomer discs and unmodified PAA discs with (white bars) and without NYS (gray bars) on freshly excised bovine vaginal mucosa according to the rotating cylinder method in VFS pH 4.2 at 37 ± 1°C. Represented values are means (±SD) of at least 3 experiments. *1 differs from PAA control ($p < 0.04$). *2 differs from PAA control + NYS ($p < 0.01$). *3 differs from PAA control ($p < 0.01$), does not differ from PAA–cysteine ($p > 0.15$). *4 differs from PAA control ($p < 0.03$), does not differ from PAA–cysteine + NYS ($p > 0.4$).

This can be explained by the formation of disulfide bonds between the thiomer and the cysteine rich subdomaines of the mucus.

One major problem of treating genitourinary infections is the short time of adhesion and furthermore the fast disintegration and washout. A prolonged residence time leads to a better patient compliance because the interval of drug application can be reduced and an increased concentration of the drug at the target site can be achieved by this close attachment. This could lead to a decreased dosage and a faster healing process. Thiomer tablets are able to swell and form disulfide bonds between their thiol groups and the thiol groups of cysteine rich subdomaines of the mucus. The more thiol groups are covalently attached the higher the TWA and the MDF are. Compared with previous mucoadhesion studies on porcine intestinal mucosa with PAA–cysteine the adhesion time on bovine vaginal mucosa is shorter.[18] Both PAA–cysteine and PAA–cysteamine as well as tablet and as gel are suitable for a vaginal delivery system because of their mucoadhesive properties.

Release Studies

Results of the release studies of thiomer gels and tablets are shown in Figure 8A and B. The PAA–cysteine 4% (w/v) gel releases of 20% of NYS within 7 days and PAA–cysteamine 4% (w/v) 30% of NYS. Compared to the PAA control gel the release is sustained. The PAA control tablets release 95% of NYS in the first 24 h. Thiomer tablets almost provided a zero-order release of NYS. Within 7 days 24% and 17% of NYS of PAA–cysteine and PAA–cysteamine tablets is released. A disadvantage of commonly used systems is the rapid removal of the inserted delivery system by the cervical mucus.[19] Therefore the drug has to be applied frequently which leads to a poor patient compliance. The analyzed gels and tablets provide a controlled release of NYS and are good formulations for a singular application which increases patient compliance.

CONCLUSION

In the present study, mucoadhesive polymers, which were synthesized by covalent attachment of L-cysteine and cysteamine, were investigated for vaginal use with NYS, because delivery systems facilitating the vaginal application of antimycotics are much in demand to increase poor patient compliance.

Each polymer conjugate has its pros and cons. The structural difference between the cysteine and cysteamine is an additional carboxyl group at

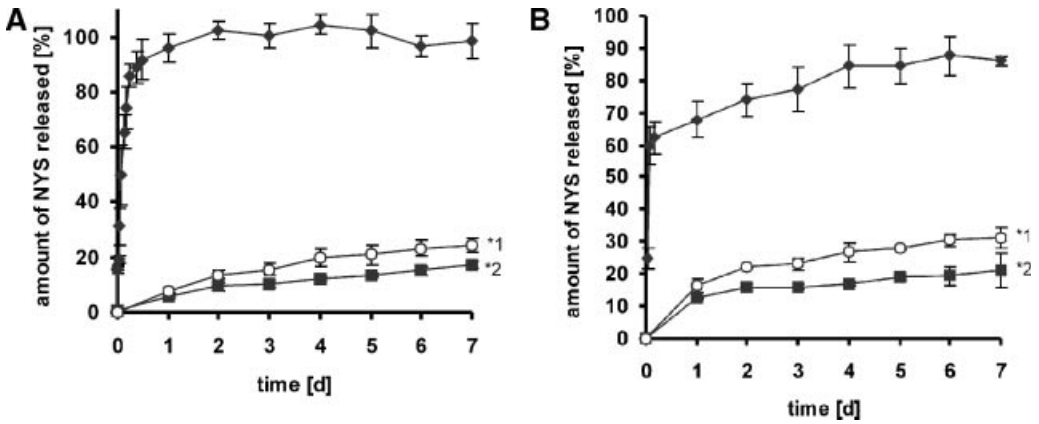

Figure 8. (A) Comparison of the release profiles of nystatin from gels containing 2% (w/v) of nystatin and 4% (w/v) of PAA control (◆), PAA–cysteine (■) and PAA–cysteamine (○), respectively. Tests were carried out in VFS pH 4.2 at $37 \pm 1°C$ for 7 days. Indicated values are means (±SD) of three experiments. *1 differs from PAA control ($p < 0.001$). *2 differs from PAA control ($p < 0.002$), differs from PAA–cysteamine ($p < 0.03$). (B) Comparison of the release profiles of nystatin from tablets containing 10 mg of nystatin and 20 mg of PAA control (◆), PAA–cysteine (■), and PAA–cysteamine (○), respectively. Tests were carried out in VFS pH 4.2 at $37 \pm 1°C$ for 7 days. Indicated values are means (±SD) of three experiments. *1 differs from PAA control ($p < 0.005$). *2 differs from PAA control ($p < 0.04$).

the cysteine molecule. This carboxyl group can also be activated by EDAC during the reaction and therefore react with another cysteine molecule. This side reaction is not possible with cysteamine. The thiomer with cysteamine should be a more uniform product.

Also because of the additional carboxyl group the cysteine can absorb more water. However this property seems to have no influence on the mucoadhesion as both in the rotating cylinder method and tensile studies displayed no significant difference to each other but a significant improvement compared to uncoupled poly(acrylic acid).

Cystein gels were more stable in rheology studies. Because of the decreasing viscosity of the PAA–cysteamine gel, the combination of NYS with PAA–cysteamine should better be applied as a tablet.

However the release was faster out of gels and tablets containing the cysteamine conjugate. The release of NYS over a time period of 1 week suggests an application with controlled release at the vaginal site.

Therefore these conjugates represent a very promising drug delivery system as they may prolong the residence at the vaginal mucosa and ensure a sustained drug release.

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