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Thiolated chitosan: Development and in vitro evaluation of an oral delivery system for acyclovir

Thomas F. Palmberger, Juliane Hombach, Andreas Bernkop-Schnürch *

Department of Pharmaceutical Technology, Institute of Pharmacy, Leopold-Franzens-Universität Innsbruck, Innrain 52, Josef Möller Haus, 6020 Innsbruck, Austria

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Abstract

The aim of the study was to develop a novel oral delivery system for the efflux pump substrate acyclovir (ACY) utilizing thiolated chitosan as excipient which is capable of inhibiting P-glycoprotein (P-gp). Three chitosan–4-thiobutylamidine (Chito–TBA) conjugates with increasing molecular mass (Chito-9.4 kDa–TBA, Chito-150 kDa–TBA and Chito-600 kDa–TBA) were synthesized and permeation studies on rat intestinal mucosa and Caco-2 monolayers were performed. Additionally, tablets comprising the conjugates and ACY were tested towards their drug release behaviour. The efflux ratio (secretory P_{app}/absorptive P_{app}) of ACY across Caco-2 monolayers was determined to be 2.5 and in presence of 100 μM verapamil 1.1 which indicates ACY as P-gp substrate. In comparison to buffer only, the transport of ACY in presence of 0.5% (m/v) unmodified chitosan, 0.5% (m/v) Chito-150 kDa–TBA and 0.5% (m/v) Chito-150 kDa–TBA with 0.5% (m/v) reduced glutathione (GSH), was 1.3-, 1.6- and 2.1-fold improved, respectively. Transport studies across Caco-2 monolayers showed that P-gp inhibition is dependent on the average molecular mass of thiolated chitosan showing following rank order: 0.5% (m/v) Chito-150 kDa–TBA/GSH > 0.5% (m/v) Chito-9.4 kDa–TBA/GSH > 0.5% (m/v) Chito-600 kDa–TBA/GSH. The higher the molecular mass of Chito–TBA was, the more sustained was the release of ACY.

Chito-150 kDa–TBA/GSH might be an appropriate sustained release drug delivery system for ACY, which is able to enhance ACY transport due to efflux pump inhibition.
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Keywords: Acyclovir; Chitosan; Thiolated polymer; P-glycoprotein; Drug release

1. Introduction

Acyclovir [9-(2-hydroxyethoxymethyl)guanine], a synthetic purine nucleoside analog derived from guanine, is a widely used agent with strong antiviral activity against herpes viruses. Acyclovir turned out to be very potent in cell culture with effective inhibitory concentrations of 1 mM or lower and is also regarded as safe from the toxicological point of view. Unfortunately, the absolute oral bioavailability having been determined to be in the range of 15–30% is considerably poor (Chiou and Barve, 1998). Thus, there have been many attempts to improve the bioavailability of acyclovir such as the development of the amino acid prodrug valacyclovir, which leads to improved bioavailability (Ganapathy et al., 1998; Kleymann, 2003). Recently, acyclovir has been suggested to be a substrate of P-glycoprotein (P-gp, MDR1) which is an efflux transporter of the ABC-binding cassette (ABC) family. The inhibition of P-gp might therefore be a promising strategy to improve intestinal uptake of acyclovir, as P-gp is – among other tissues – located at the apical side of the intestinal membrane with increasing expression levels from proximal to distal segments (Cascorbi, 2006). It has been demonstrated that in vitro acyclovir absorption could be increased due to the use of P-gp-specific inhibitors (Salama et al., 2004; Yang et al., 2004). There is generally a wide range of P-gp inhibitors available, but they are mostly small molecules which have per se a pharmacological effect. Polymers offer the advantage of not being absorbed from the gut and especially thiolated polymers – so-called thiomers – have been reported to exhibit a permeation enhancing effect for efflux pump substrates. Werle and Hoffer (2006) and Föger et al. (2006) demonstrated the transport enhancing effect of chitosan–4-thiobutylamidine (Chito–TBA) for rhodamine 123, a well-known P-gp substrate, in vitro and in vivo, respectively. Grabovac (2006) showed a similar effect for the anionic thiolated polymer poly(acrylic acid) (PAA) as well

* Corresponding author. Tel.: +43 512 507 5383; fax: +43 512 507 2933.
E-mail address: andreas.bernkop@uibk.ac.at (A. Bernkop-Schnürch).

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and demonstrated additionally that the permeation enhancing effect of thiolated PAA for efflux pump substrates is dependent on the molecular mass of the thiomer.

The objective of this study was therefore to investigate the permeation enhancing effect of thiolated chitosan on the mucosal uptake of acyclovir in dependence on the molecular mass of the utilized thiomer. Since thiolated polymers do not only offer the advantage of permeation enhancement but also the advantage of being applicable as mucoadhesive drug carrier systems, a formulation providing sustained release has been developed within this study (Bernkop-Schnürch et al., 2004a).

2. Materials and methods

2.1. Materials

Chitosan low viscous (Chito-150 kDa, average molecular mass 150 kDa, degree of deacetylation 96%), chitosan medium molecular weight (400 kDa, degree of deacetylation 84.5%) and chitosan highly viscous (Chito-600 kDa, average molecular mass 600 kDa, degree of deacetylation 75–85%) were obtained from Fluka, Buchs, Switzerland. Acyclovir was purchased from Fagron, Barsbüttel, Germany. Traut's reagent (2-iminothiolnane HCl), Ellman's reagent (DTNB, 5,5′-dithiobis(2-nitrobenzoic acid)), 2,4,6-trinitrobenzenesulfonic acid (TNBS), L-glutathione reduced form (GSH) and *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (HEPES) were obtained from Sigma–Aldrich, St. Louis, MO.

Cell culture medium was prepared by using MEM powder 9.66 g/l, 2.2 g/l sodium bicarbonate, 2 mM L-glutamine, penicillin/streptomycin solution (100 units penicillin and 0.1 mg of streptomycin per liter medium) and 20% fetal calf serum (FCS). All substances were purchased at Sigma–Aldrich, St. Louis, MO, except FCS, it was obtained from Gibco, Carlsbad, CA. Corning costar©transwell©—clear, 12 mm diameter, 0.4 μm pore size, clear polyester membranes were purchased at Corning, Acton, MA. All other chemicals were of reagent grade and obtained from Sigma, St. Louis, MO, as well.

2.2. Polymer conjugate synthesis

Chitosan with an average molecular mass of 9400 g/mol (Chito-9.4 kDa, degree of deacetylation 87%) was synthesized from chitosan medium molecular weight utilizing sodium nitrite as described previously (Mao et al., 2004; Schmitz et al., 2007). The different chitosan conjugates Chito-9.4 kDa–thiobutylamidine (Chito-9.4 kDa–TBA), Chito-150 kDa–thiobutylamidine (Chito-150 kDa–TBA) and Chito-600 kDa–thiobutylamidine (Chito-600 kDa–TBA), respectively, were synthesized according to a method described previously by our research group (Bernkop-Schnürch et al., 2003). In brief, a 1% (m/v) chitosan solution in 1% (v/v) acetic acid was stirred for 1 h. The pH was adjusted to 6.5 with NaOH. Two grams of 2-iminothiolane HCl were added. After 12 h of incubation at room temperature under continuous stirring, the resulting Chito–TBA conjugates were dialyzed first against 5 mM HCl, two times against 5 mM HCl containing 1% NaCl, against 5 mM HCl and finally against 0.4 mM HCl. Dialysis was performed under light protection at 4 °C to avoid oxidation of thiol moieties. The pH of the purified Chito–TBA conjugates was adjusted to pH 4.0. Thiomers were lyophilized by drying frozen aqueous polymer solutions at −75 °C condenser temperature and at 4×10^{-4} mbar (Virtis, Gardiner, ME). All polymer conjugates were stored at 4 °C until further use. Control polymers were prepared in the same way without submitting 2-iminothiolane to the coupling reaction.

2.3. Degree of thiolation of the polymer conjugates

The amount of free thiol groups immobilized on the polymer backbone, i.e. the degree of modification, was determined photometrically with Ellman's reagent quantifying free thiol groups (Ellman, 1958).

2.4. HPLC analysis

Samples analysis was performed via reversed-phase HPLC using a Hitachi LaChrome Elite series L2130 pump, Hitachi LaChrome Elite series L-2200 autosampler and a Hitachi LaChrome Elite L-2450 diode array detector.

Acyclovir was separated on a reversed-phase column (Nucleosil 100-5C18, 250 mm × 4 mm) at 40 °C. The isocratic mobile phase was composed of an ion pair solution (10 μM sodium phosphate dibasic and 10 μM 1-octanesulfonic acid sodium salt) and methanol in a ratio of 93:7. The pH of the mobile phase was adjusted to 2.8 with phosphoric acid and the flow rate was 1 ml/min. Injection volume was 40 μl and detection wavelength was 252 nm. The retention time was 15 min, the limit of detection was 0.1 μg/ml (signal-to-noise ratio 3) and the limit of quantification was 0.2 μg/ml (signal-to-noise ratio 9). The amount of acyclovir was calculated by interpolation from an appropriate external standard curve.

Propranolol was quantified by an HPLC method described by Panchagnula et al. (2004) and cimetidine by an HPLC method described by Iqbal et al. (2004). For both analyses a LiChrospher® 100 RP18 (5 μm) 125-4 column was used.

2.5. Permeation studies utilizing a Caco-2 cell culture monolayer system and rat intestine in Ussing type chambers

Caco-2 cells were maintained in the media described above at 95% humidity and 37 °C in an atmosphere of 5% CO_2. The media was changed daily and cells were split twice a week. The following experiments were conducted during passages 80–90. Cells were plated directly after splitting in a density of 1×10^5 cells onto the membrane inserts of 12-well plates. The cells were allowed to grow and differentiate for 24 days, during this time the media was changed every 48 h. Transepithelial electrical resistance (TEER) of the monolayers was measured with the EVOM instrument (World Precision Intruments, Sarasota, FL). Permeation studies were carried out in the transwell monolayer system, displaying a volume of 1 ml of both donor and acceptor chambers and a permeation area of 1.13 cm^2. The pH of the prepared incubation medium (cell culture medium without FCS and

the pH-indicator phenol red) was 7.4. All experiments were performed in an atmosphere of 95% O_2 and 5% CO_2 at 37 °C. After 1 h of preincubation with the incubation medium, the media of the apical or basolateral compartment was substituted by the different sample and control solutions. Over 3 h incubation time, aliquots of 200 μl were taken from the other compartment every hour, and the volume was substituted by 200 μl incubation medium pre-equilibrated at 37 °C. The amount of permeated acyclovir was determined by HPLC. Cumulative corrections were made for previously removed samples.

Right after sacrificing the rat, the small intestine (lower jejunum and ileum) was excised and strips of 1.5 cm mounted in Ussing type chambers. The chambers were displaying a volume of 1 ml (1 cm^3) of both donor and acceptor chambers and a permeation area of 0.64 cm^2. The pH of the prepared incubation medium containing 250 mM NaCl, 2.6 mM $MgSO_4$, 10 mM KCl, 40 mM glucose, and 50 mM $NaHCO_3$ buffered with 40 mM HEPES was adjusted to 6.8. All experiments were performed at 37 °C and a mixture of 5% CO_2 and 95% O_2 was continuously bubbled through the donor and acceptor compartments. After 60 min of preincubation with the artificial intestinal fluid, the medium of the apical or basolateral compartment was substituted by the different polymer conjugates (0.5%, w/v). Over 3 h of incubation time, aliquots of 200 μl were taken from the other compartment every hour, and the volume was substituted by 200 μl incubation medium pre-equilibrated at 37 °C. The amount of permeated acyclovir was determined by HPLC.

During all transport studies acyclovir was applied in a concentration of 250 μg/ml. To confirm the P-gp affinity of acyclovir, it was admitted to the apical side to investigate the transport in the absorptive direction (A → B) and to the basolateral side to investigate the transport in the secretory direction (B → A). Additionally, transport studies were performed in presence of the well-known P-gp inhibitor verapamil which was added to both sides of the rat intestinal mucosa and Caco-2 monolayer, respectively, in a concentration of 100 μM. To investigate the potential of thiolated polymers on P-gp inhibition, transport studies of acyclovir were performed in presence of 0.5% (m/v) Chito-150 kDa–TBA, 0.5% (m/v) Chito-150 kDa–TBA/0.5% GSH and unmodified Chito-150 kDa, respectively. To evaluate the influence of the average molecular mass of Chito–TBA, 0.5% (m/v) solutions of Chito-9.4 kDa–TBA, Chito-150 kDa–TBA and Chito-600 kDa–TBA, each in presence of 0.5% (m/v) GSH, have been added to acyclovir in the apical compartment. In order to assure the integrity of the Caco-2 monolayer 100 μM propranolol and 2 mM cimetidine as control markers for high and low permeability, respectively, were included in the studies.

The apparent permeability coefficients (P_{app}, cm/s) for acyclovir were calculated according to Eq. (1):

$$P_{app} = \frac{Q}{Act} \quad (1)$$

Q is the total amount permeated throughout the incubation time (mg), A the diffusion area of the Ussing type chambers (cm^2), c the initial concentration of the marker in the donor compartment (mg/cm^3), and t is the total time of the experiment (s). Transport enhancement ratios (R) were calculated from P_{app} values by Eq. (2):

$$R = \frac{P_{app}\ (\text{polymer conjugate})}{P_{app}\ (\text{buffer only})} \quad (2)$$

2.6. Release studies

The time-dependent in vitro release of acyclovir from dosage forms comprised of the three different Chito–TBA conjugates was performed as follows: In order to homogenize the polymer conjugates with acyclovir, 27 mg of the polymers were hydrated in 1% acetic acid. After addition of 3 mg of acyclovir per tablet each solution was stirred for 10 min. Thereafter the solutions were frozen at −75 °C and dried by lyophilization as described above. The different lyophilized conjugates were compressed with a compaction force of 3 kN utilizing a hydraulic press (Paul Weber, Remshalden-Grunbach, Germany). The resulting tablets were flat-faced, 5.0 mm in diameter and weighed 30 mg.

The in vitro release of the acyclovir–Chito–TBA tablets was determined by placing the tablets into 50 ml tubes containing 30 ml release medium (50 mM phosphate buffer pH 6.8). The tubes were placed into a light resistant oscillating water bath and incubated at 37 °C. Aliquots of 200 μl were withdrawn at 15 min intervals and replaced with an equal volume of release medium. Sink conditions were maintained throughout the release study, since the aqueous solubility of acyclovir was determined to be 1.3 mg/ml. The amount of acyclovir released was determined via HPLC. Cumulative corrections were made for previously removed samples.

2.7. Statistical and pharmacokinetic data analysis

Statistical data analyses were performed using the Student's t-test with $p < 0.05$ as the minimal level of significance. All values are expressed as the means ± S.D.

3. Results and discussion

3.1. Characterization of the chitosan–TBA conjugates

Thiolated chitosan has been characterized towards its swelling behaviour, mucoadhesive and cohesive properties previously (Bernkop-Schnürch et al., 2003). The isolated lyophilized conjugates Chito-9.4 kDa–TBA, Chito-150 kDa–TBA and Chito-600 kDa–TBA were determined to bear 657 ± 10, 644 ± 67 and 511 ± 63 μmol thiol groups/g polymer, respectively.

3.2. Evaluation of acyclovir as P-gp substrate

P-gp is a transporter which is located on the apical side of the intestinal membrane and it is responsible for the efflux of a wide range of xenobiotics. A characteristic feature of P-gp substrates is that they show a higher transport from the basolateral to the apical (B → A) than from the apical to the basolateral (A → B) side of an intestinal membrane. Both rat intestine and polarized Caco-2 cell monolayers have been described to express P-gp

Table 1
Comparison of the absorptive and secretory permeability coefficients (P_{app}) of acyclovir and resulting efflux ratios in presence and absence of verapamil on rat small intestine and Caco-2 monolayer, respectively (mean ± S.D., $n = 4$)

Test compound	P_{app} ($\times 10^{-6}$ cm s^{-1})		Efflux ratio [P_{app} (B → A)/P_{app} (A → B)]
	Absorptive (A → B)	Secretory (B → A)	
Rat small intestine			
Buffer	9.06 ± 1.21	20.56 ± 1.79	2.3
Verapamil	14.18 ± 1.88	15.33 ± 0.77	1.1
Caco-2 cells			
Buffer	0.56 ± 0.04	1.41 ± 0.11	2.5
Verapamil	0.98 ± 0.08	1.07 ± 0.11	1.1

in considerable amounts (Hunter et al., 1993; Valenzuela et al., 2004). Thus, the transport of acyclovir from A → B and B → A has been performed across rat intestine and Caco-2 cell monolayers, respectively, to ensure that acyclovir is a P-gp substrate. The determined apparent permeability coefficients (P_{app}) are listed in Table 1. The secretory transport of acyclovir was 2.5-fold higher in case of the Caco-2 monolayer and 2.3-fold higher in case of rat small intestine in comparison to the absorptive transport. Additionally, the transport of acyclovir in presence of verapamil, a well-known inhibitor of P-gp, has been tested. Due to the inhibitory effect of verapamil, the absorptive and secretory transport of acyclovir was not significantly different. TEER of the Caco-2 monolayers was measured during the transport experiments and was determined to be 380 ± 35 Ω cm^2 after the experiment for acyclovir in buffer and 365 ± 40 Ω cm^2 for acyclovir with verapamil. TEER did not significantly change during the experiment (data not shown).

To evaluate the monolayer viability permeation studies were performed with the high permeable marker propranolol and the low permeable marker cimetidine. The P_{app} of propranolol is with $19.90 \pm 0.59 \times 10^{-6}$ cm s^{-1} much higher than the P_{app} of acyclovir (Table 2). Propranolol is in the range of the P_{app} of $13.5 \pm 0.34 \times 10^{-6}$ cm s^{-1} (Asano et al., 2003), the P_{app} of $34.43 \pm 2.26 \times 10^{-6}$ cm s^{-1} (Gres et al., 1998) and the P_{app} of $36.6 \pm 0.9 \times 10^{-6}$ cm s^{-1} (Markowska et al., 2001) reported previously. Cimetidine with a P_{app} of $1.26 \pm 0.11 \times 10^{-6}$ cm s^{-1} is low permeable being in good agreement with data described by Markowska et al. (2001).

Table 2
Comparison of the in vitro apparent permeability coefficients (P_{app}) of acyclovir and improvement ratios in presence of indicated test compounds for absorptive transport (A → B) (mean ± S.D., $n = 4$)

Test compound	P_{app} ($\times 10^{-6}$ cm s^{-1})	Improvement ratio R
Rat intestine		
Buffer	9.06 ± 1.21	–
Chito-150 kDa	12.11 ± 0.42	1.3
Chito-150 kDa–TBA	14.77 ± 1.66	1.6
Chito-150 kDa–TBA + GSH	18.61 ± 1.52	2.1
Caco-2 cells		
Buffer	0.56 ± 0.04	–
Chito-9.4 kDa–TBA + GSH	0.93 ± 0.05	1.7
Chito-150 kDa–TBA + GSH	1.54 ± 0.16	2.8
Chito-600 kDa–TBA + GSH	0.92 ± 0.10	1.6
Cimetidine	1.26 ± 0.11	
Propranolol	19.90 ± 0.59	

Werle and Hoffer (2006) determined for rhodamine 123, a specific P-gp substrate, an efflux ratio of 2.8 which declined to an efflux ratio of 1.0 due to addition of verapamil. Within this study, similar efflux ratios for acyclovir could be determined which suggest acyclovir as P-gp substrate. De Vrueh et al. (1998) also detected a three-fold higher secretory flux than absorptive flux for acyclovir across Caco-2 cell monolayers.

3.3. Efflux pump inhibition studies on rat intestinal mucosa

The influence of Chito-150 kDa–TBA with and without GSH and its unmodified control polymer on the transport of acyclovir across rat intestinal mucosa has been evaluated in Ussing type chambers. Results of the in vitro permeation studies are shown in Table 2. The transport of acyclovir could be enhanced by a factor 1.3 due to the addition of Chito-150 kDa. In the presence of Chito-150 kDa–TBA, the transport was 1.6-fold improved and due to the addition of GSH, it was even further improved. A 2.1-fold transport improvement for acyclovir was achieved in presence of 0.5% Chito-150 kDa–TBA in combination with 0.5% GSH which leads to a P_{app} value of $18.61 \pm 1.52 \times 10^{-6}$ cm s^{-1}. The P_{app} of acyclovir in buffer was determined to be $9.06 \pm 1.21 \times 10^{-6}$ cm s^{-1}, which is in good correlation with Žakelj et al. (2004) who detected a P_{app}-value of 9.6×10^{-6} cm s^{-1} in HEPES buffer. Besides P-gp inhibition, additional mechanisms of transport enhancement, such as opening tight junctions might explain the improved absorption due to the Chito-150 kDa–TBA/GSH system. The opening of tight junctions is especially relevant for hydrophilic compounds which are absorbed via paracellular transport. Thiolated chitosan/GSH systems were repeatedly described to facilitate paracellular drug uptake (Bernkop-Schnürch et al., 2004b). Clausen et al. (2002) described GSH to play a crucial role, because it interacts via disulfide bonds with the enzyme tyrosine phosphatase (PTP), which regulates the opening status of tight junctions. The thiolated polymer is necessary in this system to prevent GSH from oxidation on the surface of the mucosa. The mechanism of P-gp inhibition of thiolated chitosan is not believed to be based on competitive inhibition, like it is reported for small molecules which are substrates or inhibitors of P-gp and ATP-depletion (Lo and Huang, 2000). Additionally, thiomers are not absorbed from the gut and therefore they cannot inhibit ATP which is necessary for P-gp activity by binding intracellular Ca^{2+} which is necessary for ATP activity. It is possible that thiolated chitosan inhibits P-gp by forming disulfide bonds with cysteine subunits of P-gp. P-gp exhibits 12 transmembrane regions forming a channel through which its substrates are transported. Two of these regions, namely 2 and 11, exhibit on positions 137 and 956, respectively, a cysteine subunit. Thiomers might enter this channel and interact with these cysteine subunits located within the channel (Bernkop-Schnürch and Grabovac, 2006).

A notable difference between the P_{app} values of Caco-2 cells and rat intestine can be seen although the P_{app} values of both Caco-2 cells and rat intestine are similar to those reported in literature and despite the difference in permeability rates the improvement ratios and efflux ratios determined in this study are in good agreement. This can be explained by the tighter epithelium of the colonic Caco-2 cells which leads to a low passive permeability. In contrast, rat ileum is more leaky and the passive paracellular flow dominates (Artursson et al., 1993).

3.4. Efflux pump inhibition studies on Caco-2 monolayers

As shown in Fig. 1, the permeation enhancing effect on acyclovir of thiolated chitosan could also be demonstrated on Caco-2 cell monolayers. The improvement ratio achieved by the Chito-150 kDa–TBA/GSH system in comparison to control was 2.8. The P_{app}-value of acyclovir transport in buffer solution across Caco-2 monolayers was determined to be $0.56 \pm 0.04 \times 10^{-6}$ cm s^{-1} (Table 2), which is in good correlation with previously published studies showing P_{app} values of 0.25×10^{-6} (Corti et al., 2006) and 0.6×10^{-6} cm s^{-1} (Han et al., 1998). All these P_{app} values are below 0.7 which is an indicator for poorly absorbable drugs.

Caco-2 cell monolayers became a widely accepted in vitro model membrane for intestinal drug absorption and several publications have already shown that human drug absorption can be predicted from data obtained by transport studies on Caco-2 monolayers. According to Artursson and Karlsson (1991) who compared bioavailability in humans and P_{app} values from Caco-2 transport studies, drugs with P_{app} values higher than 1×10^{-6} cm s^{-1} are completely absorbed in humans. If the prediction model of Yamashita et al. (2000) is applied to the results obtained within this study, acyclovir in buffer solution would be absorbed in humans to around 50% (experimental determined bioavailability: 20% (15–30%)) and in presence of the thiomer/GSH system even to 100%. If the discrepancy between predicted and experimental determined bioavailability is considered, the thiomer/GSH system would lead to bioavailability of around 60% in humans.

In Fig. 1 it is additionally shown that the length of the polymeric chains plays an important role. The chain length – in case of non cross-linked polymers – correlating with the molecular mass, has an influence on the flexibility and therefore the ability of the polymer to form entanglements with mucus (Leitner et al., 2003). In order to achieve transport enhancement of polymers, the ability of a polymer to interpenetrate the mucus layer is important. This fact suggests small polymer chains to use, however, small molecules are too short to exhibit the maximum feasible extent of interpenetration (Caramella et al., 1994). Additionally, very small polymers are not able to form a cohesive three-dimensional network, which is necessary for mucoadhesion and therefore a prolonged effect of inhibition. The results obtained within this study correlate with Grabovac (2006) who investigated the influence of the molecular mass of anionic poly(acrylic acid) on efflux inhibition. They determined thiolated poly(acrylic acids) with an average molecular mass of 100 and 200 kDa, respectively, as most suitable for efflux pump inhibition which is in good correlation with this study. Schipper et al. (1996) showed also that the molecular weight of different chitosans has an influence on transport improvement.

3.5. Release studies

In Fig. 2 the release profile of acyclovir from tablets based on the three different thiolated polymers is shown. All release

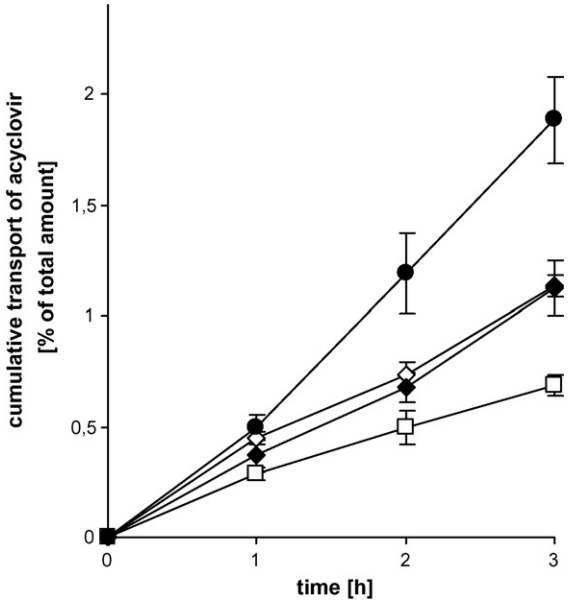

Fig. 1. Transport studies of acyclovir across Caco-2 cell monolayers. Effect of 0.5% (m/v) Chito-9.4 kDa–TBA + 0.5% (m/v) GSH (◊), 0.5% (m/v) Chito-150 kDa–TBA + 0.5% (m/v) GSH (●), 0.5% (m/v) Chito-600 kDa–TBA + 0.5% (m/v) GSH (♦) in comparison to control (□) (mean ± S.D., $n = 4$).

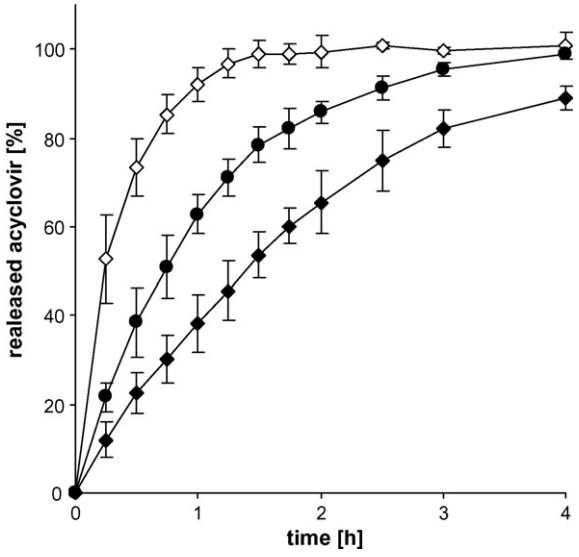

Fig. 2. Release of acyclovir from tablets composed of Chito-9.4 kDa–TBA (◊), Chito-150 kDa–TBA (●) and Chito-600 kDa–TBA (♦). The 30 mg tablets were incubated with 30 ml release medium (phosphate buffer pH 6.8) at 37 °C (mean ± S.D., $n = 4$).

profiles display a similar profile with the drug being constantly released. Within 4 h 100% of acyclovir was released from the delivery system composed of Chito-150 kDa–TBA and within 1.5 h 100% of acyclovir was released from the delivery system composed of Chito-9.4 kDa–TBA. Chito-600 kDa–TBA released acyclovir comparatively most slowly and almost zero-order release kinetics could thereby be observed. The dependence of the release profile of acyclovir on the average molecular mass of the thiomers applied, might be explained by the ability of polymeric chains to form a cohesive three-dimensional network. Obviously, the longer the chains are, the better the cohesive properties of thiolated chitosan are.

4. Conclusion

Within the present study, further evidence has been provided for acyclovir as efflux pump substrate. Thiolated chitosan was shown to enhance the transport of acyclovir across rat intestinal mucosa and Caco-2 cell monolayers. Especially Chito-150 kDa–TBA in combination with GSH exhibited significantly improved drug uptake. It could be demonstrated that thiol moieties are essential for effective P-gp inhibition. Additionally, the inhibitory effect of thiolated chitosan on P-gp was described to be dependent on the average molecular mass of the applied chitosan and Chito-150 kDa–TBA could be identified to have the most appropriate polymeric chain length. Based on the improved uptake of the representative P-gp substrate acyclovir, thiolated chitosan 150 kDa might be suggested as effective polymeric excipient in order to improve the intestinal uptake of P-gp substrates.

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