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Design and Evaluation of a New Gastrointestinal Mucoadhesive Patch System Containing Chitosan-Glutathione

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Within this study, a novel gastrointestinal patch system was developed and investigated regarding water-absorbing capacity, adhesive properties, in vitro release, unidirectional release and permeation enhancing effect. Water uptake studies revealed that the weight of patch systems with Ch-GSH increased about 44.5 ± 2.3 mg (127%) after 90 min. This patch system remained even after 180 h on the mucosa and released 49.7 ± 0.7% of FD_4 within 8 h. A 2.5-fold higher transport of FD_4 can be obtained in contrast to control. In conclusion this patch system could be an interesting possibility for the transport through the intestinal mucosa of macromolecules which will normally be degraded in the intestinal tract.

Keywords patch system; chitosan-glutathione; FD_4, controlled release

INTRODUCTION

A couple of different gastrointestinal patch systems were developed to improve the overall oral bioavailability of large molecules that can currently be delivered only by parenteral route (Still, 2002). For example Eaimtrakarn et al. designed a patch system which comprises a three layer system. In vivo tests with dogs showed that this oral delivery system increased area under the plasma concentration versus time curve and mean residence time of fluoresceine isothiocyanate-dextran in comparison to conventional tablets (Eaimtrakarn et al., 2003). In another study Shen et al. established a novel method to prepare patches. Intestinal patches were prepared by sandwiching a film of crosslinked bovine serum albumin microspheres between a film of ethyl cellulose and Carbopol/pectin. Permeation studies with rat intestine showed that patches were effective in delivering model drugs across the intestine (Shen & Mitragotri, 2002).

These entire patch systems provide bioadhesion, drug protection and unidirectional release. This combination of function could improve the overall oral bioavailability of drugs which are poorly available when administered orally. However the major causes of the low oral bioavailability of macromolecular drugs are generally due to luminal enzymatic hydrolysis and low membrane permeability (Tao & Desai, 2005). Within the last decade Bernkop-Schnürch et al. showed that thiolated polymers exhibit improved mucoadhesive, controlled release, permeation enhancing, and enzyme inhibitory properties (Bernkop-Schnürch, Schwarz & Steininger, 1999). The so called thiomers represent a new generation of mucoadhesive and permeation enhancing polymers. Permeation studies with hydrophilic model drugs across intestinal mucosa demonstrated that the combination of thiolated polymers with GSH led to a significantly improved drug uptake in the presence of thiomers (Clausen, Kast, Bernkop-Schnürch, 2002).

In order to combine the both promising strategies, patch systems and thiomers, it was the purpose of this study to use Ch-GSH as multifunctional layer with the protective coating of a patch system.

As shown in Figure 1 the system consists of four layered films: the backing layer is made of water-insoluble polymer ethyl cellulose (EC) inducing unidirectional release of drug. The surface layer consists of Eudragit L100, an enteric pH-sensitive polymer, providing protection of the formulation during transport through the stomach. The layer between middle and surface layer contains lactose, starch, and confectioners' sugar to separate the cationic drug layer from the anionic surface layer. The middle layer contains FD_4 as a model drug, the mucoadhesive polymer Ch-GSH and unbound GSH. FD_4 was chosen as model drug being poorly absorbed from the GI-tract and having the same dimension like many proteins such as insulin.

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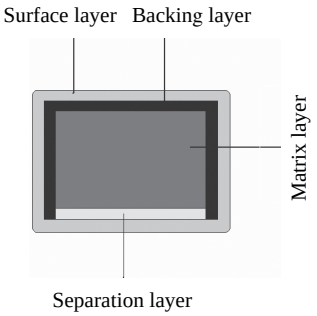

FIGURE 1. Constitution of the patch system which consists of four layered films: the backing layer is made of ethyl cellulose (EC). The surface layer is made of Eudragit L100. The layer between middle and surface layer is made of lactose, starch, and confectioners' sugar. The middle layer contains FD_4 as a model drug, the mucoadhesive polymer Ch-GSH and unbound GSH.

MATERIALS AND METHODS

Materials

Chitosan (medium molecular mass: 400 kDa; degree of deacetylation: 83–85%) was obtained from Fluka Chemie (Buchs, Switzerland). L-glutathione reduced form (GSH), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC), N-(2-hydroxyethyl) piperazine-N-(2-ethanesulfonic acid) (HEPES), 5, 5-dithiobis(2-nitrobenzoic acid), ethylcellulose (EC), FD_4 (medium molecular mass: 4 kDa) and minimum essential medium (MEM) were all purchased from Sigma (St. Louis, MO). Eudragit L100 was obtained from Röhm Pharma (Darmstadt, Germany). N-Hydroxysuccinimide (NHS) was obtained from Acros (Geel, Belgium) and FCS from Gibco (NR. 26140-079). All chemicals were of analytical grade. Male Sprague Dawley rats were purchased from the medical university of Vienna (Austria). Permeation studies were performed with rats weighed between 258 and 307g.

Synthesis of Chitosan-Glutathione Conjugates

The covalent attachment of GSH to chitosan was achieved via the formation of amide bonds between carboxylic acid moieties of GSH and amine groups of chitosan.

First, 1 g of chitosan was hydrated in 8 mL of 1 M HCl and then dissolved by the addition of demineralized water in order to obtain a 1% (w/v) polymer solution. The pH was adjusted to 6.0 by the addition of 5 M NaOH. Afterwards, 5 g of GSH in 10 mL demineralized water was added under stirring. Then, EDAC dissolved in 5 mL demineralized water was added in a final concentration of 200 mM. Thereafter, 200 mM NHS dissolved in 5 mL demineralized water was added into the reaction mixture under vigorous stirring. The pH was readjusted to 6.0 with 5 M NaOH. The reaction mixture was incubated for 7 h at room temperature under permanent stirring. The resulting polymer conjugate was dialysed in tubings (molecular weight cut-off 12 kDa) first against 5 mM HCl, twice against 5 mM HCl containing 1% NaCl, and finally two times against 1 mM HCl. Finally, the frozen aqueous polymer solutions (samples, controls and complex) were lyophilized at −50°C and 0.01 mbar (Lyolab B; Inula, Austria) and stored at 4°C until further use (Kafedjiiski, Föger, Werle & Bernkop-Schnürch, 2005).

Preparation of the Patch System and Control Tablets

The granulate for the separation layer was made as described by Lieberman & Lachman (1980). After filling 15 mg of the granulate for the surface layer in the tablet press, 20 mg lyophilized Ch-GSH conjugate was added, in which FD_4 (6% w/w) and GSH (5% w/w) were embedded and compressed into 35 mg, 5.0 mm diameter flat-faced tablets (single punch eccentric press-Paul Weber Maschinenbau, Modell 10, Remshalden-Grunbach, Germany). Pressure of 8 kN was kept constant during the preparation of all tablets. For control tablets, 15 mg of granulate for the separation layer, was filled in the tablet press and thereafter 20 mg lyophilized chitosan was added, in which FD_4 (6% w/w) and GSH (5% w/w) were embedded and compressed as described above. Then, they were placed on a support and coated with a solution of 11% w/v EC in acetone on all sides except one. Acetone was evaporated at room temperature. Utilizing this procedure an EC layer of about 30 μm thickness was obtained. For this type of formulation the synonym "coated tablet" will be used for the rest of the manuscript. Thereafter, all tablets were enteric coated with Eudragit L100 (Röhm, Darmstadt, Germany). For enteric coating, the tablets were dipped in an 11% (w/v) Eudragit L100 solution of methylenchloride/methanol (4:1) and air-dried. This coating procedure was repeated three times. Utilizing this procedure a Eudragit layer of about 50 μm thickness was obtained. For this type of formulation the synonym "patch system" is used throughout the manuscript. Tablets, coated tablets and patch systems were stored at 4°C until further use. Compositions of the dosage forms used for in vitro studies were shown in Table 1.

Uniformity of Mass

Uniformity of tablet mass was determined according to the method described in the European Pharmacopoeia. For the uniformity of mass tests, patch systems were weighed on a Sartorius balance (type BC BL 100).

Crushing Strength

The matrix tablets that matched the projected drug release profiles were characterized in terms of their crushing strength using a Roche tablet crushing strength tester model 65. The test was performed with twelve tablets. The mean value and the standard deviation were calculated.

TABLE 1
Compositions of the Dosage Forms Used for In Vitro Studies

Ingredients	Tablet	Coated Tablet	Patch System
FD_4 (theoretical load)	1.2 mg	1.2 mg	1.2 mg
FD_4 (actual drugload)	1.2 mg	0.9 mg	0.9 mg
Ch-GSH	17.8 mg	17.8 mg	17.8 mg
GSH	1 mg	1 mg	1 mg
Separation layer	15 mg	15 mg	15 mg
EC layer	–	+	+
Surface layer	–	–	+

Ingredients	Control Tablet	Control Coated Tablet	Control Patch System
FD_4 (theoretical load)	1.2 mg	1.2 mg	1.2 mg
FD_4 (actual load)	1.2 mg	1.1 mg	1.1 mg
Chitosan	17.8 mg	17.8 mg	17.8 mg
GSH	1 mg	1 mg	1 mg
Separation layer	15 mg	15 mg	15 mg
EC layer	–	+	+
Surface layer	–	–	+

FTIR Spectroscopy

Fourier transform infrared (FTIR) spectra were recorded with a Bruker IFS 25 spectrometer (Bruker Analytische Messtechnik GmbH, Ettlingen, D). Samples were scanned as potassium bromide disks over a range of 4000 to 400 cm^{-1} at an instrument resolution of $2\,cm^{-1}$ (64 scans per spectrum).

In Vitro Release Studies of Test Compound

The release rate of FD_4 from tablets and patch systems was analysed in vitro. Patch systems were placed in a beaker containing 7.5 mL 0.1 M HCl pH 1. After 2 h the pH was adjusted to 6.8 following the pH change dissolution procedure specified in USP for enteric-coated particles: 2 h of exposure to 7.5 mL of 0.1M HCl followed by testing in 10 mL of pH 6.8 phosphate buffer, the pH being adjusted with 2.5 mL of 0.2 M tribasic sodium phosphate solution. 1.2 mg of FD_4 was incorporated in our patch system and the volume of the release medium was about 10 mL. In this manner sink conditions could be maintained with a solubility of 25 mg/mL water of the model compound. The amount of FD_4 released from the patch into the solution was quantified by measuring fluorescence (Fluostar Galaxy) at wavelength of 492 nm (extinction) and 520 nm (emission) (BMG Labtechnologies) and was calculated from an according standard curve obtained by solutions with increasing concentrations of FD_4.

Determination of the Unidirectional Release of Model Compound from Patches

Release of FD_4 from patch systems was carried out in HEPES buffer (1.45 g NaCl, 0.42 g $NaHCO_3$, 0.72 g glucose, 59.4 mg KCl, 59.4 mg $MgSO_4$ and 953.2 mg HEPES in 100 mL demin. H_2O) at pH 6.8. To distinguish drug release from the mucoadhesive and the backing side of the patch system, the patch systems were placed in Ussing-type chambers. The Ussing-type chamber was comprised of two chambers placed side by side with an opening provided between the chambers of about 0.64 cm^2. A patch system was added on the intestinal mucosa between the two sides of the chamber and each side was filled with 1 mL HEPES buffer solution pH 6.8. The amount of FD_4 released from either side of the patch into the solution was quantified as described above.

Permeation Studies on Rat Intestine

Permeation studies were carried out in Ussing type chambers displaying a volume of 1 mL of both, donor and acceptor chamber and a permeation area of 0.64 cm^2. HEPES buffer (pH 6.8) was used for the permeation studies.

Right after sacrificing the rat, the first 15 cm of the small intestine were excised and mounted in the Ussing chamber. All experiments were performed in an atmosphere of 95% O_2 and 5% CO_2 at 37°C. Tablets, control tablets, coated tablets or coated control tablets were attached to the apical side of the intestinal mucosa. The length of the small intestine mounted onto the Ussing chamber was about 1 cm^2. FD_4 was used as model compound. Over 4 h incubation time, aliquots of 200 µL were taken from the acceptor compartment in 60 min intervals and the volume was substituted by 200 µL incubation medium preequilibrated at 37°C. The amount of permeated FD_4 was determined using a microtitration plate reader (Fluostar Galaxy, Offenburg, Germany). Cumulative corrections were made for the previously removed samples. The apparent permeability coefficients (P_{app}) for FD_4 were calculated according to the following equation:

$$Papp = Q/(A*c*t)$$

where Papp is the apparent permeability coefficient (cm/s), Q is the total amount permeated throughout the incubation time (µg), A is the diffusion area of the Ussing chamber (cm^2), c is the initial concentration of the marker in the donor compartment ($\mu g/cm^3$), and t is the total time of the experiment (s).

Caco-2 Cell Permeation

Caco-2 cell monolayer (passage number 85) was grown onto 12 well Transwell polycarbonate membranes (Transwell®, Costar, 0.4 µm pore size, 12 mm diameter) according to the protocol as described by Sattler, Schäfer, Schneider, Hölzl and Lehr (1997). The cells were cultured in MEM medium

supplemented with 20% fetal calf serum (FCS). The culture medium was exchanged every other day and the cells were stored in a 5% CO_2-incubator at 37°C. Permeation studies were performed with Caco-2 cell monolayers cultured for 24 days. Caco-2 cell monolayers with trans-epithelial electrical resistance (TEER) values in the range of 500 to 600 Ω cm^2 were used for permeation studies. Prior to all experiments, each Caco-2 cell monolayer was washed with phosphate buffer saline (pH 6.8). Then 1 mL of HEPES buffer was added to the apical and 1.5 mL to the basolateral chambers and after a 30 min equilibration period in 5% CO_2 incubator, TEER was measured to ensure integrity of the Caco-2 cell monolayers. The transport experiments were performed by replacing the medium on both sides with HEPES buffer adjusted to pH 6.8. The HEPES solution was replaced by the same buffer containing Ch-GSH or chitosan, GSH and the hydrophilic marker FD_4. TEER was measured every hour and samples of 50 µL were taken every hour from the acceptor compartment and replaced by fresh buffer.

The integrity of the Caco-2 cell monolayer was evaluated by measuring the TEER using an EVOM® (World Precision Instruments Inc., Sarasota Florida) connected with a pair of electrodes. The TEER was measured throughout the transport studies. After completion of the permeation studies, transport medium was removed carefully and Caco-2 cell monolayers were rinsed with phosphate buffer saline (pH 6.8) and the culture medium was applied on the monolayers. The Caco-2 cell monolayers were allowed to regenerate for 24 h in the CO_2-incubator.

Trypan Blue-Exclusion Test

After treatment of a Caco-2 cell monolayer with Ch-GSH and FD_4, chitosan and FD_4 or FD_4 the apical and basal sides were washed with MEM medium supplemented with 20% FCS, to wash out all test compounds. The cell monolayers were further incubated at 37°C for 160 min with fresh MEM medium supplemented with 20% FCS on the apical and basal sides, respectively. After the medium on both sides had been exhausted, 100 µL of 0.4% (w/v) trypan blue aqueous solution was added to the apical chamber. The cell monolayers were then incubated for 2 min and gently washed twice with phosphate-buffer saline. The Caco-2 cell monolayers with the filter were carefully removed from the Transwell insert and placed into a new test tube. The trypan blue was then extracted from the cell monolayer by adding 1 mL of a mixture of acetone and 0.5% (w/v) Na_2SO_4 at the ratio of 7 to 3 and leaving overnight at room temperature. After 5 min sonication the material was centrifuged at 3000 rev min^{-1} for 15 min. The supernatant was dried and redissolved in 200 µL of the solvent like described above. The absorption of 150 µL of the redissolved solution was measured at 560 nm with a microplate reader (Fluostar Galaxy).

Evaluation of the Swelling Behavior

The water-absorbing capacity was determined by a gravimetric method (Bernkop-Schnürch, Guggi & Pinter, 2004). Test tablets were fixed to a needle and incubated in a 0.1 M phosphate buffer pH 6.8 at 37°C. At scheduled time intervals the hydrated test tablets were taken out of the incubation medium, excess water was removed and the amount of water uptake was determined gravimetrically on a Sartorius balance (type BC BL 100).

In Vitro Mucoadhesion Studies with the Rotating Cylinder Method

In vitro Mucoadhesion was tested using "Apparatus 6" of the United States Pharmacopeia (USP) [USP, 23rd edition, <711> "Dissolution"] which is identical to the "Paddle Apparatus" of the European Pharmacopeia (Ph.Eur.). An Erweka DT 700 (Erweka GmbH, Heusenstamm, Germany) dissolution tester was used. Stirrers in cylinder form are provided as part of the mucoadhesion tester. Each vessel was filled with 900 mL of 0.1 M phosphate buffer pH 6.8 at 37°C ± 0.5°C. Rotational speeds of the cylinders were 125 min^{-1} in various phases of product testing. Coated tablets and control coated tablets were attached by hand to a freshly excised intestinal porcine mucosa, which has been attached to a stainless steel cylinder (diameter: 4.4 cm; height 5.1 cm). The detachment of the test tablets was determined visually during an observation time of 180 h (Bernkop-Schnürch & Kast, 2001).

Statistical Data Analysis

Statistical data analysis were performed using the students test with $p < 0.05$ as the minimal level of significance.

RESULTS

Formulation of the Patch System

Intestinal patches utilized in this study were at about 5 mm in diameter and 1 mm in thickness and consisted of a mucoadhesive matrix in which FD_4 and GSH were dispersed. The mucoadhesive matrix consisted of Ch-GSH. It was synthesized by the amide bond formation between glycine carboxylic acid groups of GSH and amine groups of chitosan as described by Kafedjiiski, Föger et al. (2005). The average mass of the patches was: 35.4 ± 1.51 mg (*SD*, $n = 20$). Results from the testing of the crushing strength were: mean value of 44.5 ± 1.51 N (*SD*, $n = 10$) and friability studies showed 0.7 ± 0.1% of abrasion (*SD*, $n = 18$). The FTIR spectra of Ch-GSH show significant peaks at 1670 and 3370 cm^{-1} in comparison to FTIR spectra of chitosan (Figure 2). Amides in the solid state are characterized by asymmetric and symmetric NH_2 stretching frequencies near 3370 and 3190 cm^{-1} and a strong band at 1670–1620 cm^{-1}, which is usually a doublet near 1660 and 1630 cm^{-1} involving C=O stretch and NH_2 deformation (Colthup, Daly & Wiberley, 1990). These results are in good

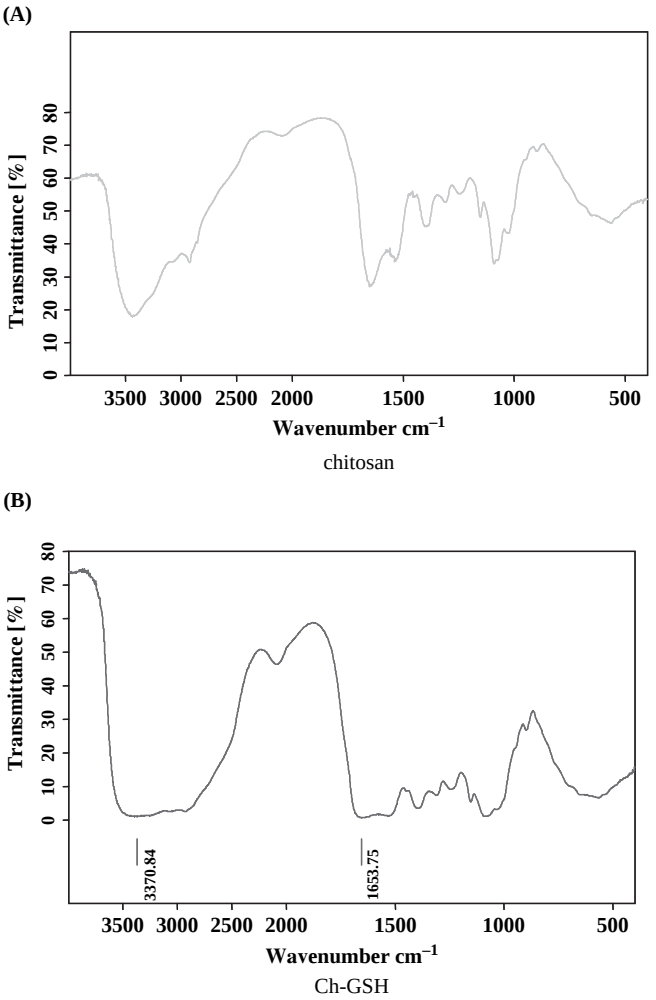

FIGURE 2. Comparison of the FTIR spectra of chitosan (Figure 2A) and Ch-GSH (Figure 2B).

accordance with the theory of the covalent attachment of GSH to chitosan via the formation of amide bonds between carboxylic acid moieties of GSH and amine groups of chitosan.

In Vitro Release Studies of Test Compound

In vitro studies showed that FD_4 was released from patches over a period longer than 8 h (Figure 3). The release study was conducted in gastric environment for 2 h. Then the pH was adjusted to 6.8. No possible leakage of the drug in the gastric medium was found. The result was added. After changing the pH to 6.8 49.7 ± 0.7% of FD_4 was released within 6 h from the mucoadhesive side of the patches with Ch-GSH as matrix layer in contrary to 71.82 ± 2.39% for patches with the unmodified chitosan as matrix layer. 86.51 ± 4.46% of FD_4 from tablets with Ch-GSH as matrix layer was released and 99.0 ± 2.81% from tablets with chitosan as matrix layer. A sustained release of FD_4 for several h was observed (Figure 3) for patch systems

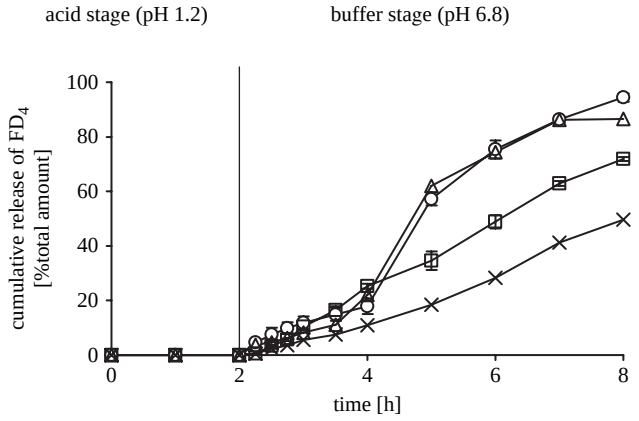

FIGURE 3. Release profile of FD_4 from patch system made out of Ch-GSH (x) in comparison to control tablets comprising Ch-GSH (Δ) and release profile of FD_4 from patch system made out of chitosan (□) in comparison with control tablets made out of chitosan (○). Studies were performed with phosphate buffer solution with a pH of 6.8. Indicated values are means (± *SD*) of at least three experiments.

based on Ch-GSH. The release behavior of the patches could be explained with their designed constitution as drug delivery system and using the thiomer Ch-GSH as a carrier of the primary matrix. The polymer matrix probably combines two major types' of mechanisms for drug release: controlled diffusion and swelling (Kafedjiiski, Krauland, Hoffer, Bernkop-Schnürch, 2005).

Determination of the Unidirectional Release of Test Compound from Patches

The application of the patch system in comparison to tablets caused a higher concentration on the basolateral side of the Ussing chambers. After 3 h 46 ± 5% of FD_4 was liberated from Ch-GSH tablets in comparison to 61 ± 2% from chitosan tablets and 5 ± 2% from patch systems. One reason might be caused by the fact that the marker leaves from all sides from the tablets whereas FD_4 only leaves from one side from the patch system. Results are shown in Figure 4a.

Permeation Studies on Rat Intestine

Permeation studies carried out using rat intestine showed that there was significant difference between coated tablets with Ch-GSH and chitosan. If a patch system comprising Ch-GSH/GSH was utilized a 2.1-fold higher transport of FD_4 could be reached in comparison to patch systems with chitosan and unbound GSH. The combination of thiolated chitosan with unbound GSH shows a strong permeation enhancing effect in comparison to chitosan. Results are shown in Figure 4b.

The P_{app} values calculated for the patch system with Ch-GSH/GSH, control patch systems with chitosan/GSH and FD_4 were determined to be 8.8×10^{-6}cm/s, 4.6×10^{-6} cm/s and 6.2×10^{-6} cm/s, respectively.

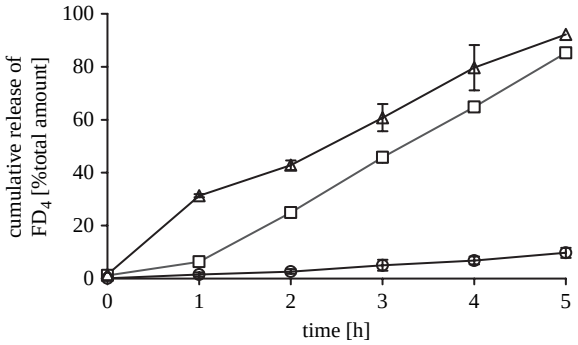

FIGURE 4A. Determination of the unidirectional release of model compound in using chamber on the basolateral side from coated tablets with CH-GSH (○) in comparison with control tablets (Δ) and tablets (□). Studies were performed with phosphate buffer solution with a pH of 6.8. Indicated values are means (± *SD*) of at least three experiments.

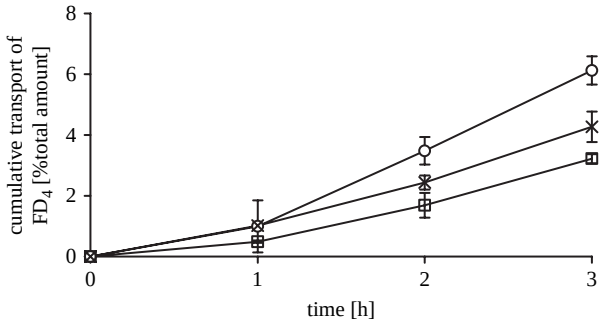

FIGURE 4B. Permeation enhancing effect on the apical side of coated tablets across freshly excised small intestinal mucosa with Ch-GSH (○) in comparison with control tablets (×) and tablets (□). Studies were performed with phosphate buffer solution with a pH of 6.8. Indicated values are means (± *SD*) of at least three experiments.

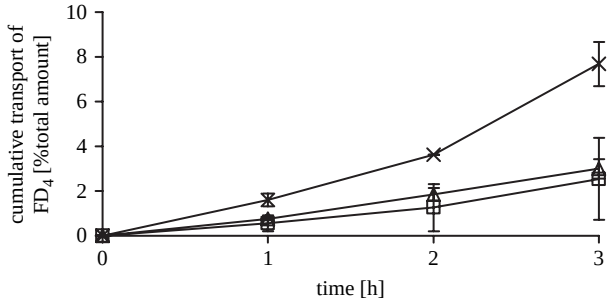

FIGURE 5. Permeation enhancing effect of unmodified chitosan, 0.5% GSH and FD_4 (□) or Ch-GSH with 0.5% GSH (w/v) and FD_4 (x) in comparison with FD_4 (Δ) across CaCo-2 Cells. Indicated values are means (±*SD*) of at least three experiments.

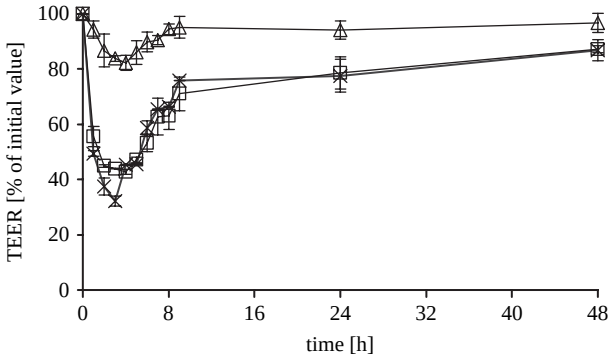

FIGURE 6. Reversibility of Ch-GSH effects on monolayer permeability of unmodified chitosan, 0.5% GSH and FD_4 (□) or Ch-GSH with 0.5% GSH (w/v) and FD_4 (x) in comparison with FD_4 (Δ) across CaCo-2 cells. Indicated values are means (± *SD*) of at least three experiments.

Caco-2 Cell Permeation

The effect on the absorption rate of Ch-GSH in combination with unbound GSH was studied. Ch-GSH in combination with unbound GSH caused a 2.0-fold higher transport of FD_4 in comparison to FD_4 alone. Results are shown in Figure 5. TEER was measured during and after permeation studies with Caco-2 cell monolayers to ensure integrity of the membrane. TEER of the membrane covered with Ch-GSH in combination with GSH and model compound rapidly decreased instead of TEER of the membrane only covered with model compound. 4–5 h after treatment TEER nearly recovered completely and after 24 h TEER of all membranes recovered full. Results are shown in Figure 6.

Trypan Blue-Exclusion Test

Plasma membrane damage was evaluated by uptake of trypan blue into the cell. The result for the positive control, 0.1% (w/v) sodium dodecylsulphate (SDS) treatment, was approximately nine times higher than that for the control monolayers. Results from treatment with Ch-GSH with FD_4 and chitosan with FD_4 were similar to that of the control, suggesting no damage to the plasma membrane.

Swelling Behavior

As shown in Figure 7 water uptake studies revealed that patch systems showed a minor swelling behavior in comparison to tablets without coating. The results for the patch systems comprising Ch-GSH and patch systems consisted of chitosan were different. The weight from patch systems with Ch-GSH increased to about 44.5 ± 2.3 mg (127%) after 90 min and from patch systems with chitosan increased to about 73.1 ± 8.4 mg (209%) after 90 min. The weight from tablets with Ch-GSH increased to about 139.3 ± 6.3 mg (398%) and from tablets with chitosan increased to about 147.8 ± 7.9 mg (422%). At the end of the experiment, no erosion or dissolving of the tablets and the patch systems was observed. The slow swelling process favours the high cohesive properties of the conjugate.

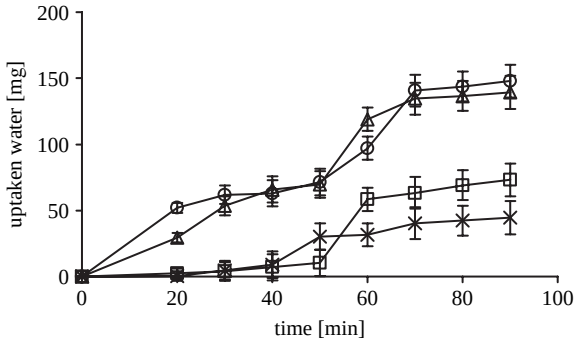

FIGURE 7. Swelling behavior of control patch system (□) and patch system (x) in comparison with control tablets (○) and tablets (Δ) in 0.1 M phosphate buffer solution pH 6.8 at 37°C; indicated values are means (± *SD*) of at least three experiments.

In Vitro Mucoadhesion Studies

Results of mucoadhesion studies performed with the rotating cylinder method are shown in Figure 8. Since this test system takes also into account of the cohesiveness of the polymers, it is supposed to be closer to in vivo conditions than simple tensile studies. In this connection patch systems with Ch-GSH were compared to patch systems with chitosan as a carrier matrix. Thereby, obtained results demonstrated improved adhesive properties due to the immobilization of thiol groups. Patch systems based on Ch-GSH conjugate remained attached to the mucosa even after 180 h of incubation. In contrast, the corresponding control made out of chitosan detached from mucosa within 24 h. Therefore a 7.5 fold higher mucoadhesion could be achieved from coated tablets with Ch-GSH as matrix layer in comparison to control coated tablets with chitosan as matrix layer.

DISCUSSION

Within this study a new formulation was developed to improve the oral bioavailability of large molecules. After selection of the composition and the process the conformity of the patch system with the European pharmacopoeia was evaluated. Uniformity of mass, crushing strength and friability were according to the guidelines and were reproducible.

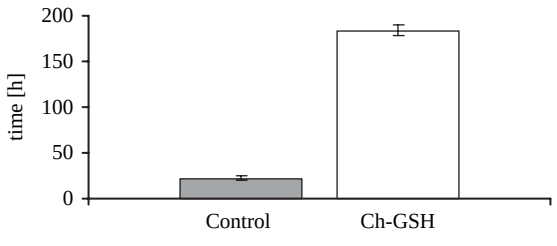

FIGURE 8. Comparison of the adhesion time of coated tablets and coated control tablets on the rotating cylinder. Indicated values are means (± *SD*) of at least three experiments.

The release profile of drugs is dependent upon the nature of the matrix and the complex interaction between the process of swelling, diffusion and erosion. In polymers loaded with drugs, various release profiles can be obtained depending on the relative magnitude of the rate of polymer swelling to the rate of drug diffusion. Therefore Ch-GSH was chosen as matrix layer because Bernkop-Schnürch et al. (2004) showed in a preliminary study that thiomers exhibit good release behavior and an even pseudo order release rate with various model compounds could be achieved. The delivery system designed within this study can also guarantee such a sustained release and indicates a pseudo zero order release kinetic. Due to this controlled and sustained release profile, objectionable interactions between the thiolated polymer matrix and FD_4 can be excluded. The release rates of patch systems were always lower compared with the tablets. This was caused by the presence of an ethyl cellulose layer for the protection of the agent against enzymatic degradation. The presence of EC layer minimized the release from the edges as well as from the back side of the patch into the intestinal lumen and also might prevent the enzyme penetration into the patch. Additionally the unidirectional release caused by the EC layer resulted in increased local drug concentrations, which enhance the absorption efficiency and minimized adverse effects (Whitehead, Zancong & Mitragotri, 2004). Furthermore premature drug degradation in the stomach was avoided by the surface layer, prepared with Eudragit L100. Tao and Desai (2005) described that proteins and large molecules are often unstable and degraded prior to absorption when exposed to the acidic environment of the stomach and enzymatic hydrolysis in the intestine. This problem leads to unacceptably low oral bioavailability of the drug. The presence of an enteric surface layer will protect the drug from the harsh g.i. condition and will be dissolved in the intestine. There upon, the patch system will bind on to the mucosa.

The efficacy of such enteric coated mucoadhesive delivery systems being based on the anionic and cationic mucoadhesive thiolated polymers could meanwhile be demonstrated in various in vivo studies. In one study, the plasma calcium level of rats was reduced by the oral administration of calcitonin being embedded in a thiolated chitosan carrier matrix, which was enteric coated (Guggi, Kast, Bernkop-Schnürch, 2003).

The present study demonstrated a 2.1-fold higher transport of FD_4 if a patch system with Ch-GSH in combination with GSH was utilized in comparison to a patch system with unmodified chitosan. The likely mechanism being responsible for this improved permeation enhancing effect is specified by Clausen et al. (2002). These results agree with permeation studies across Caco-2 cell monolayer. It has been considered that Caco-2 cells adhere more tightly to each other than the small intestinal cells. This might explain the lower permeability of that membrane to hydrophilic drugs that permeate the intestinal membrane mainly through the tight junctional route

(Tanaka et al., 1995). A time dependent decrease of the TEER under the influence of Ch-GSH in combination with GSH followed by an increase of marker transport was observed, indicating a loosening of the tightness of intercellular junctions, i.e. the opening of the paracellular route across the epithelium for normally low-absorbable compounds (Anderberg, Nyström & Artursson, 1992). The loss of epithelial barrier function is paralleled by a loss of cellular polarization and differentiation, but the cells are obviously not irreversibly damaged and are still capable to redifferentiate after 24 hours. This corresponds with the results from the trypan blue-exclusion test. Results shows, that Ch-GSH and chitosan have in combination with FD_4 no toxic effect on Caco-2 cell monolayers.

The absence of toxic effects is all the more important in connection with the intensified contact of thiomers with the gastrointestinal mucosa. The intensified contact with the mucosa should provide the prerequisite for an increased epithelial permeability and prolonged residence time in the gastrointestinal gut (Roldo, Hornof, Calicet & Bernkop-Schnürch, 2004). The average contact time for mucosal routes of drug delivery behaves about 3 hours for the small intestine, with intermediate time for the other routes, thereby resulting in a significant barrier to drug delivery (Roldo et al., 2004). A 7.5 fold higher mucoadhesion could be achieved from coated tablets with Ch-GSH as matrix layer in comparison to control coated tablets with chitosan as matrix layer. The main mechanism of mucoadhesion of thiomers is based on the formation of disulfide bonds between the polymer and cysteine-rich subdomains of mucus glycoproteins (Bernkop-Schnürch, Kast & Guggi, 2003). The primary role in the exchange reaction plays the activity of the thiol groups of the conjugate. Their activity is determined from the chemical structure and the corresponding pK_a, which is favourable for the formation of sufficient concentration of thiol anions in the physiological medium. The selection of glutathione ligand was based upon its thiol pK_a value (8.7) and the rate of solubility. Whether the improved mucoadhesive properties of the delivery system described here is sufficient to guarantee a prolonged residence time in the small intestine will be a subject of further studies. Mucoadhesion is directly associated with swelling behavior. The swelling behavior of mucoadhesive polymers has considerable influence on their adhesive properties and cohesiveness (Steininger & Bernkop-Schnürch, 2000). The hydration theory postulates that mucoadhesive polymers take water from the underlying mucosal tissue by absorbing, swelling and capillary effects, leading to a considerably strong adhesion (Kafedjiiski, Krauland et al., 2005). An over hydrated form that loses its mucoadhesive properties before reaching the target is undesired. Therefore covering of tablets with surface layer has not only the advantage of protection against enzymatic degradation but also protection from being over hydrated. Results showed that coated tablets have a minor swelling behavior compared to tablets without coating. In addition, good mucoadhesive properties were obtained for coated tablets.

In conclusion good mucoadhesive and permeation enhancing features in combination with protective coating were determined for this patch system. According to the obtained results this patch system might be an interesting possibility for the transport of various macromolecules which will normally be degraded in the gastrointestinal tract.

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