Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 296 (2005) 103–111
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Development of a mucoadhesive and permeation enhancing buccal delivery system for PACAP (pituitary adenylate cyclase-activating polypeptide)

Nina Langoth [a,*], Jochen Kalbe [b], Andreas Bernkop-Schnürch [c]

[a] *Thiomatrix GmbH, Mitterweg 24, A-6020 Innsbruck, Austria*
[b] *High Tech Private Equity GmbH, Steinstraße 20, 40212 Düsseldorf, Germany*
[c] *Department of Pharmaceutical Technology, Institute of Pharmacy, Leopold-Franzens-University Innsbruck, Innrain 52, 6020 Innsbruck, Austria*

Received 29 December 2004; received in revised form 28 February 2005; accepted 2 March 2005
Available online 8 April 2005

Abstract

The buccal mucosa providing direct entry into the systemic circulation appears to be a potential site for the delivery of PACAP (pituitary adenylate cyclase-activating polypeptide), a new therapeutic agent in the treatment of type 2 diabetes. In order to reach a sufficient buccal bioavailability a drug delivery system with strong permeation enhancing and mucoadhesive properties is needed. In this study the enhancing effect of a strongly mucoadhesive chitosan–thioglycolic acid (TGA) conjugate in combination with reduced glutathione (GSH) on the permeation of PACAP across the buccal mucosa was investigated. The apparent permeability coefficient (P_{app}) of PACAP in buffer only was $(5.7 \pm 3.1) \times 10^{-8}$, while in the presence of chitosan–TGA conjugate (1%) a P_{app} of $(20.0 \pm 3.4) \times 10^{-8}$ was achieved. The combination of chitosan–TGA (1%) with GSH (2%) led to an improvement of the P_{app} up to $(57.3 \pm 31.7) \times 10^{-8}$. Release studies of PACAP demonstrated that a controlled release can be provided from tablets consisting of chitosan–TGA at a pH of 5, whereas more than twice as much was released from chitosan–TGA tablets pH 4. According to the combination of permeation enhancing properties, controlled drug release and the mucoadhesive character, chitosan–TGA conjugates represent a promising tool for the buccal administration of PACAP.

Keywords: Chitosan; Chitosan–thioglycolic acid conjugate; Buccal drug delivery; Permeation enhancement; PACAP

1. Introduction

Type 2 diabetes is a common disorder affecting up to 45% of individuals older than 65 years (Wagman and Nuss, 2001). The treatment is often limited by numerous problems encountered with

* Corresponding author. Tel.: +43 512 89 00 46; fax: +43 512 89 00 46 15.
E-mail address: n.langoth@thiomatrix.com (N. Langoth).

0378-5173/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2005.03.007

so far pursued therapeutic strategies. Sulfonylurea therapy, for instance, has the disadvantage of causing hypoglycemia and high primary and secondary failure rates (Harrower, 2000). Treatment with biguanides has also a high primary and secondary failure rate and the potential to cause lactic acidosis (Calabrese et al., 2002). Furthermore, thiazolidinediones can cause weight gain and liver toxicity (Rosak, 2002). Ultimately the majority of diabetics will require insulin treatment, a therapy that produces significant bouts of hypoglycemia when attempting to maintain tight control of plasma glucose levels. Hence, new treatments in order to retain or enhance glucose-dependent insulin secretion are needed. Recently, a novel peptide drug for treatment of type 2 diabetes, so called PACAP (pituitary adenylate cyclase-activating polypeptide), has been evaluated (Fig. 1). PACAP is expected to offer a unique advantage for type 2 diabetic patients because of its potential to induce insulin synthesis and secretion from pancreatic beta cells in a glucose-dependent manner. PACAP combines the potency of insulin without risking overdose and hypoglycemia (Yada et al., 2000).

Because of its relatively short elimination half-life period of about 4 min on the one hand and the need of a permanent blood level of the drug on the other hand (Filipsson et al., 1997; Xiao et al., 2001), from the drug delivery point of view, implantable pumps or inserted depot formulations are needed. Because of the inconvenience of these delivery systems, non-invasive dosage forms would be preferred. Their development, however, seems much more challenging as the poor mucosal absorption of peptides leads to a comparatively very poor bioavailability. Buccal drug delivery systems are thereby representing a promising alternative to parenteral administration. The good accessibility of this absorption site and the high acceptance by the patients favor the buccal mucosa as a site for systemic drug delivery (Veuillez et al., 2001).

Chitosan was chosen as drug carrier matrix, since it is known to act as a mucoadhesive biopolymer for mucosal drug delivery systems (Bernkop-Schnürch, 2000; Senel et al., 2000). Its favorable biological properties such as non-toxicity, biocompatibility and biodegradability make chitosan a promising candidate for a safe buccal drug delivery system (Senel and Hincal, 2001). In addition, a sustained release of peptide drugs out of this polymer can be provided (Singla and Chawala, 2001). Moreover, chitosan exhibits a permeation enhancing effect (Schipper et al., 1997).

Recently, it has been shown that the thiolation of chitosan, by the covalent attachment of thioglycolic acid (TGA), leads to a strongly improved mucoadhesion compared to unmodified chitosan (Roldo et al., 2004). The chemical structure of chitosan–TGA is shown in Fig. 2. As it has been demonstrated by our research group (Clausen et al., 2002), that the immobilization of thiol groups on polymers leads also to an improved permeation enhancing effect being mediated by GSH (L-γ-glutamyl-L-cysteinylglycine), drug uptake in the presence of chitosan–TGA conjugate and GSH on the buccal tissue was evaluated as well.

Accordingly, the purpose of the present study was to develop a buccal drug delivery system for PACAP providing sufficient adhesion of the formulation on the mucosa (objective I), a sustained release of the

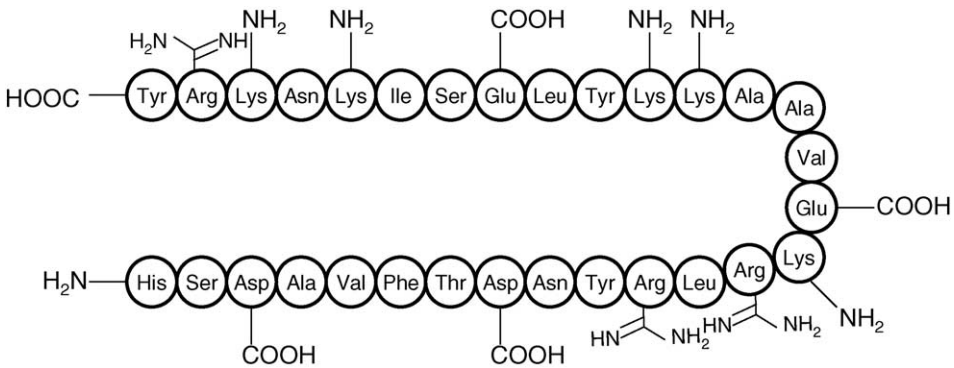

Fig. 1. Amino acid sequence of PACAP, mass of 3741 Da.

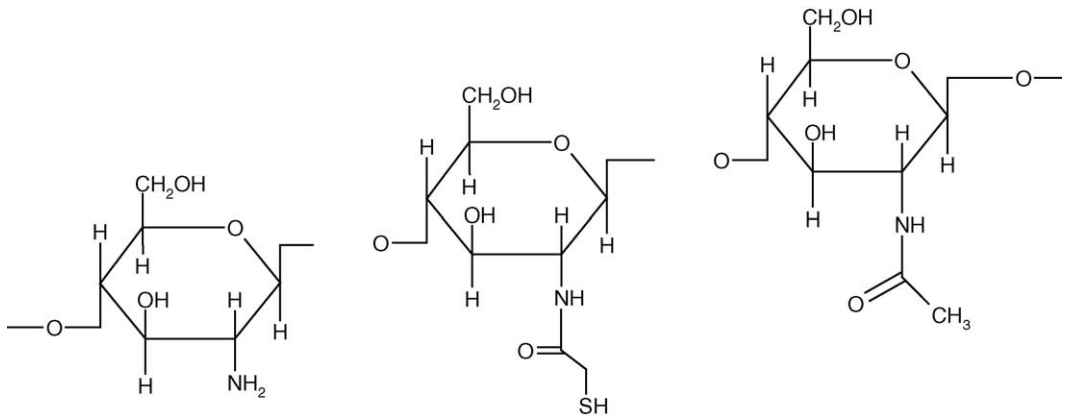

Fig. 2. Presumptive substructures of chitosan–thioglycolic acid conjugates.

therapeutic agent (objective II) and a permeation enhancing effect (objective III).

2. Materials and methods

2.1. Synthesis of chitosan–thioglycolic acid conjugates

Chitosan–thioglycolic acid conjugates (chitosan–TGA) were synthesized by a method described previously our research group (Kast and Bernkop-Schnürch, 2001). The immobilization of thiol groups on the surface of the cationic polymer chitosan was achieved by the covalent attachment of thioglycolic acid to the primary amino groups of chitosan via an amide bond formation. The coupling reaction was mediated by a carbodiimide.

In brief, to 50 ml of a 1% solution of chitosan hydrochloride (molecular mass: ∼150 kDa; degree of deacetylation: 83–85%; Fluka Chemie, Buchs, Switzerland) 500 mg of thioglycolic acid (TGA; Sigma, St. Louis, MO) were added. Thereafter, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC; Sigma, St. Louis, MO) dissolved in 1 ml demineralized water was added in a final concentration of 125 mM. During the coupling reaction the pH was kept constant at 5 by adding 1 M HCl. The reaction mixture was incubated for 3 h at room temperature under continuous stirring. In order to isolate the chitosan–TGA conjugate the polymer solution was dialyzed. Samples prepared in the same way but omitting EDAC served as controls. Thereafter, samples and controls were lyophilized at −30 °C and 0.01 mbar (Christ Beta 1–8 K; Germany) and stored at 4 °C until further use.

2.2. Determination of thiol group content

The degree of modification, i.e. the amount of thiol groups immobilized on chitosan, was determined spectrophotometrically with Ellman's reagent. First, 0.50 mg of the conjugate were hydrated in 250 μl of demineralized water. Then, 250 μl of 0.5 M phosphate buffer pH 8.0 and 500 μl of Ellman's reagent (3 mg 5,5′-dithiobis(2-nitrobenzoic acid); Sigma, St. Louis, MO, dissolved in 10 ml of 0.5 M phosphate buffer pH 8.0) were added. Samples were incubated for 3 h at room temperature. The supernatant was separated from the precipitated polymer by centrifugation (24 000 × *g*, 5 min). Thereafter 300 μl of the supernatant were transferred to a microtitration plate and the absorbance was measured at a wavelength of 450 nm with a microtitration plate reader (Anthos reader 2001; Salzburg, Austria). Thioglycolic acid standards were used to calculate the amount of thiol groups immobilized on the polymer.

2.3. Tissue preparation

In these studies porcine buccal mucosa was used, because porcine and human buccal epithelia are similar in certain important parameters, i.e. permeability, barrier lipid composition, histology and ultrastructural

organization (Wertz and Squier, 1991). Buccal tissue from pigs weighing 70–100 kg, obtained freshly from a local slaughterhouse, was used at least within 4 h after sacrificing the animals. Most of the underlying tissue was removed from the mucosa with surgical scissors. The epithelium was separated from most of connective tissue with an electro-dermatome (Aesculap® Accu Dermatome, Germany). The buccal tissue was dermatomed with a thickness of 500 μm.

2.4. Permeation studies

Dermatomed buccal mucosa was mounted in Ussing chambers with a diffusion area of 0.64 cm^2 and a compartment volume of 1 ml. All experiments were performed in an atmosphere of 95% O_2 and 5% CO_2 at 37 °C. Prior to the experiment, the acceptor chamber was filled with 40 mM Bis–Tris buffer (bis[2-hydroxyethyl]imino-tris[hydroxymethyl]methane) pH 6.8, and the donor chamber with either chitosan–TGA (1% m/v) containing 2% (m/v) of the reduced form of GSH, or chitosan–TGA (1% w/v), or unmodified chitosan (1% m/v). The polymers were dissolved in 40 mM Bis–Tris buffer pH 6.8. After an equilibration period of about 20 min, 100 μl of buffer on the donor side were replaced with a PACAP solution to reach a concentration of 1 mg of PACAP/ml. Every 30 min 150 μl samples were withdrawn from the acceptor chamber and replaced by 150 μl buffer, equilibrated at 37 °C. The amount of permeated PACAP was determined using HPLC as described below. Cumulative corrections were made for the previously removed samples. The apparent permeability coefficients (P_{app}) for PACAP were calculated according to the equation $P_{app} = Q/(A \times c \times t)$, where P_{app} is the apparent permeability coefficient (cm/s), Q the total amount permeated within the incubation time (μg), A the diffusion area of the Ussing chamber (cm^2), c the initial concentration of the marker in the donor compartment (μg/cm^3), and t is the total time of the experiment (s).

2.5. Viability studies

After the permeation studies the medium was removed from the donor chamber and incubated in 1 ml of trypan blue dye. Microscopic investigations were performed after a staining time of 15 min.

2.6. HPLC analysis of PACAP

Analysis of samples was carried out by reversed phase HPLC using a Perkin-Elmer series 200 LC pump (Norwalk, CT, USA), Perkin-Elmer 200 series auto sampler with a 20 μl injection loop and a diode array detector (Perkin-Elmer 235C). PACAP and its degradation products were separated on a precolumn (Nucleosil 100-5C18, 40 mm × 4 mm) and a C_{18}-column (Nucleosil 100-5C18, 250 mm × 4 mm) at 40 °C. Gradient elution was performed as follows: flow rate 1 ml/min; 0–22 min; linear gradient from 90% A/10% B to 10% A/90% B (eluent A: 0.1% trifluoroacetic acid in water; eluent B: 90% acetonitrile and 10% of 0.1% trifluoroacetic acid in water). The absorbance of the peptide was detected at 220 nm. The amount of PACAP was calculated by interpolation from an appropriate external standard curve.

2.7. Evaluation of the reducing properties of chitosan–TGA conjugate against GSSG

First, 50 mg of the polymer-conjugate were hydrated in 5 ml of 40 mM Bis–Tris buffer pH 6.8. After the polymer was hydrated completely, 100 mg of oxidized glutathione (GSSG; Sigma, St. Louis, MO) was added. This mixture was then placed on a water permeable membrane serving as the bottom of a plastic tube with a diameter of 30 mm. The tube was set in a vessel (diameter 35 mm) containing 15 ml of the 40 mM Bis–Tris buffer and 300 mg of GSSG to provide an equilibrium within the system. The test was carried out at 37 °C, and every 30 min samples of 150 μl were withdrawn for a period of 6 h and replaced by buffer. Samples were evaluated with Ellman's reagent sensitive to free thiol groups.

2.8. Release studies

In order to homogenize the polymer and the polymer-conjugate, respectively, with PACAP, the lyophilized polymers were hydrated in a sufficient amount of demineralized water. An adequate amount of PACAP was added and the pH of these polymer/PACAP solutions was adjusted and measured by a pH meter. Mixtures were frozen and lyophilized. Polymer mixtures containing 1 mg of PACAP and either 29 mg of the chitosan–TGA conjugate or

29 mg of unmodified chitosan were compressed into 5.0 mm diameter flat faced discs with a thickness of 2 mm (tablet machine: Hanseaten Type EI, Hamburg, Germany). The compaction pressure was kept constant during the preparation of all tablets. The hardness of the tablets was 210 ± 20N ($n = 10$; Pharma Test PTB 311, Hainburg, Germany).

The in vitro release rate from these drug delivery systems was analyzed in the following way. The dosage forms were placed in 25 ml beakers (Schott, Duran 25 ml, G) containing 10 ml of release medium (50 mM phosphate buffer, pH 6.8). Due to the small size of the tablets and the low amount of PACAP a release medium of 10 ml was used to ensure that the amount of released PACAP could be detected. The vessels were closed, placed on an oscillating water bath (GFL 1092; 100 rev./min) and incubated at 37 ± 0.5 °C. Sink conditions were maintained throughout all these studies. Aliquots of 150 μl were withdrawn in 1 h intervals and replaced with an equal volume of release medium preequilibrated at 37 °C. Released PACAP was assayed by HPLC as described above.

2.9. Data analysis

Statistical data analysis was performed using the *t*-test with $p < 0.05$ as the minimal level of significance. All experiments were performed at least three times.

3. Results

3.1. Synthesis of chitosan–thioglycolic acid conjugates

TGA was attached covalently to the primary amino groups of chitosan under the formation of amide bonds as shown in Fig. 2. The carboxylic acid moieties of TGA were activated by EDAC forming an *O*-acylurea derivative as intermediate product, which reacts with the primary amino groups of chitosan. The efficacy of the purification method for the resulting polymer–TGA conjugates could be verified by controls, which were prepared in exactly the same way as the polymer-conjugates, but omitting EDAC during the coupling reaction, exhibiting a negligible amount of thiol groups. The lyophilized chitosan–TGA conjugates appeared as white, odorless powder of fibrous structure. The purified chitosan–TGA conjugate displayed 175.63 ± 8.22 μM sulfhydryl groups per gram polymer.

3.2. Viability studies

After permeation studies the tissue was put into a trypan blue staining solution. Microscopic investigations after an incubation period of 15 min demonstrated, that the viability of the buccal tissue was still guaranteed, as no blue colour was detectable within the cells.

3.3. Permeation studies

The permeation enhancing effect of chitosan, chitosan–TGA conjugate (1% m/v), GSH (2% m/v), and chitosan–TGA conjugate in combination with GSH, respectively, on the absorption of PACAP was evaluated. The permeability coefficients for PACAP alone and in the presence of the permeation enhancers are shown in Table 1. Chitosan did not lead to a significantly improved transport of PACAP across the buccal tissue. In contrast the addition of chitosan–TGA conjugate caused a 3.51-fold increase in the permeation of PACAP in comparison to the control (Fig. 3). In studies with the chitosan–TGA conjugate in combination with GSH the permeability coefficient increased even 10-fold compared to PACAP alone, whereas in the presence of GSH without the polymer the enhancement ratio was 2.75 (Fig. 4).

Table 1
Effect of chitosan, GSH, chitosan–TGA and chitosan–TGA + GSH, respectively, on the buccal permeation of PACAP and the corresponding enhancement ratios (enhancement ratio = P_{app}(sample)/P_{app}(control))

Test system	Apparent permeability (±S.D.) ($\times 10^{-8}$ cm/s)	Enhancement ratio
Control	5.70 ± 3.31	1
Chitosan 1% (m/w)	8.50 ± 1.34	1.5
GSH 2% (m/w)	15.69 ± 3.06[a]	2.8
Chitosan–TGA 1% (m/w)	20.04 ± 3.40[a]	3.5
Chitosan–TGA 1% (m/w) + GSH 2% (m/w)	57.34 ± 31.70[a]	10.1

Indicated values are means (±S.D., $n = 3$–4).
[a] Significantly different from the control.

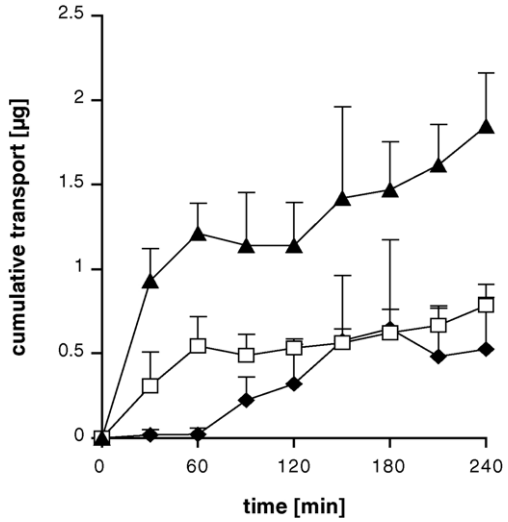

Fig. 3. Permeation profile of PACAP across buccal mucosa of pigs. Control without polymer (♦); 1% (w/v) chitosan (□); 1% (w/v) chitosan–TGA (▲) (means ± S.D., $n = 3$).

3.4. Evaluation of the reducing effect of chitosan–TGA conjugate

The polymer was incubated with GSSG to evaluate the potential of chitosan–TGA conjugate to reduce GSSG to GSH. As shown in Fig. 5 3.80 ± 0.34 μg of GSH could be detected within 6 h. Control experiments of GSSG incubated with unmodified polymer or without polymer showed no detectable thiol groups within the incubation period.

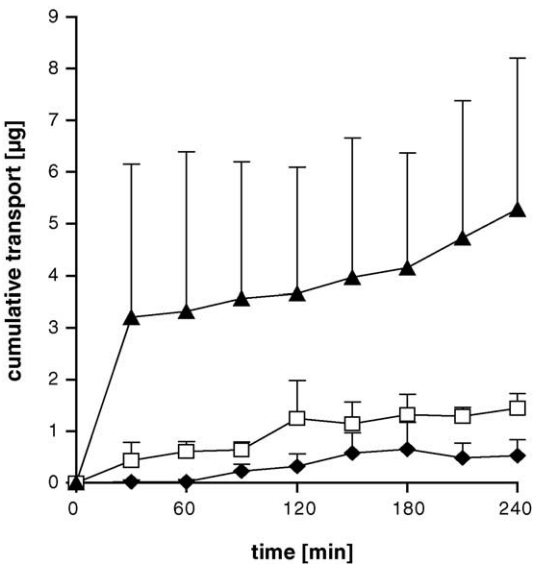

Fig. 4. Transport of PACAP across buccal mucosa of pigs. Control without polymer and glutathione (♦); 2% (w/v) GSH (□); 1% (w/v) chitosan–TGA and 2% GSH (▲) (means ± S.D., $n = 3$).

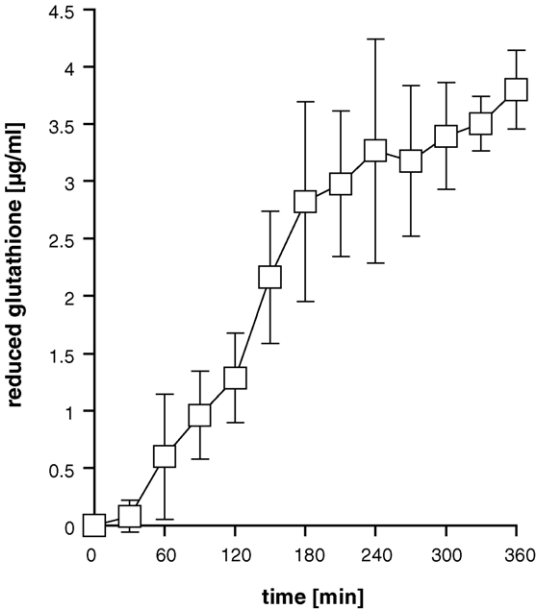

Fig. 5. Increase of reduced glutathione (□; GSH) by incubation of 2% (m/v) oxidized glutathione (GSSG) with 1% (m/v) chitosan–TGA at 37 °C, pH 6.8. Indicated values are means ± S.D. of at least three experiments.

3.5. Release studies

In preliminary studies the release of PACAP from tablets consisting of polycarbophil–cysteine conjugate (Bernkop-Schnürch et al., 1999) has been investigated. However, due to ionic interactions between the cationic charged drug and the anionic character of the polycarbophil–cysteine conjugate no drug release could be observed (data not shown).

Studies carried out with tablets based on unmodified chitosan showed a rapid drug release (Fig. 6). Using tablets based on chitosan–TGA conjugate with a pH of 4, the release of PACAP could be lowered for the first 6 h, but after 8 h nearly the same drug amount was released compared to the chitosan tablets. A controlled drug release was achieved by shifting the pH of the polymer matrix from 4 to 5. After 8 h 45.9 ± 4.4%

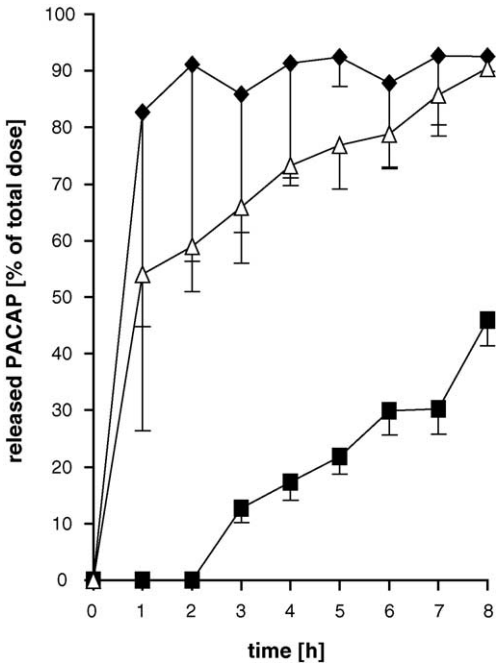

Fig. 6. Release profile of PACAP from tablets based on chitosan pH 5 (♦), chitosan–TGA pH 4 (△), and chitosan–TGA pH 5 (■). Studies were carried out in 50 mM phosphate buffer pH 6.8 at 37 °C. Indicated values are means (±S.D.) of at least three experiments.

of PACAP had been released from the chitosan–TGA tablets with a pH of 5, whereas more than twice as much was released from the chitosan–TGA tablets pH 4. The tablets did not disintegrate and showed no erosion during the experiment.

4. Discussion

4.1. Mucoadhesion

The covalent attachment of thioglycolic acid to the cationic polymer chitosan leads to conjugates exhibiting an up to 10-fold improved mucoadhesion, which was shown in former investigations (Kast and Bernkop-Schnürch, 2001). The derivative combines two different mechanisms of mucoadhesion: ionic interactions between the cationic groups of chitosan and the anionic moieties provided by sialic acid and sulfonic acid substructures within the mucus layer (a), and the formation of disulfide bonds due to the introduction of thiol groups (b) (Bernkop-Schnürch, 2000).

4.2. Permeation enhancement

One of the major disadvantages associated with buccal drug delivery is the low flux across the mucosa which results in low drug bioavailability. In general, for peptide drugs, permeation across the buccal epithelium is thought to be through paracellular route by passive diffusion, and the intercellular connections in buccal tissue are characterized by desmosomes and tight junctions (Rathbone et al., 1994). Unmodified chitosan is able to enhance the paracellular absorption, which is important for the transport of hydrophilic compounds, such as peptides and proteins, across the membrane (Ranaldi et al., 2002). The underlying mechanism for this permeation enhancing effect seems to be based on the positive charges of the polymer, which interact with the cell membrane, resulting in a structural reorganization of tight junction associated proteins (Schipper et al., 1997). Due to the covalent attachment of thioglycolic acid the rate of transported PACAP through the buccal mucosa could be improved significantly. Compared to unmodified chitosan the permeation enhancing effect of chitosan–TGA was 2.6-fold increased. This observation might suggest the idea that the free thiol groups of the polymer are able to reduce oxidized glutathione, which is situated on the surface of the buccal mucosa. Reduced glutathione seems to inhibit the protein–tyrosine–phosphatase, which leads in turn the phosphorylation of occludin. This phosphorylation causes the opening of tight junctions and hence the transport of large molecules across the membrane (Barrett et al., 1999; Staddon et al., 1995). Recently it was shown by our research group, that thiolated polycarbophil in combination with GSH led to a 2.9-fold increase in the transport of sodium fluorescein across the small intestinal mucosa of guinea pigs (Clausen et al., 2002). Within this study it was shown that chitosan–TGA conjugate in combination with GSH leads to a 2.9-fold increase in the transport of PACAP across the buccal mucosa of pigs in comparison to chitosan–TGA alone. The activity of GSH seems thereby to be dependent on the amount of free thiol groups on the polymer. This observation may be related to the results evaluating the activity of chitosan–TGA conjugate to reduce GSSG. The amount of GSH on the absorption membrane will be increased, and the higher GSH concentration leads to an improved opening of the tight junctions. Because of this likely synergistic

effect of the combination of chitosan–TGA and GSH, a permeation enhancement ratio of 10.06 was reached.

4.3. Enzyme inhibition

The amount of active drug reaching the systemic circulation intact depends also on the stability of the peptide towards peptidases present on the site of administration. Recently, it could be shown that thiolated polycarbophil is able to stabilize peptides against degradation by mucosal enzymes (Walker et al., 2001). Accordingly, a similar effect might be expected for chitosan–TGA conjugates, but has to be verified by further studies.

4.4. Controlled drug release

Another advantage of chitosan–TGA in drug delivery systems is a controlled drug release, which can be reached by the polymer used as carrier matrix. A controlled drug release of PACAP over a period of 8 h was provided by incorporating PACAP in chitosan–TGA. The effect of the pH of the chitosan–TGA conjugate on the release of PACAP was evaluated. The release of PACAP can be controlled by adjusting the pH value within the polymer carrier matrix to a certain value. The retention of PACAP in the three-dimensional network of the cationic polymer is thereby achieved by electrostatic interactions between chitosan–TGA and anionic substructures of PACAP. At pH 5 PACAP exhibits more negative than positive charges. Hence, a controlled release of PACAP can be reached, because of ionic interactions between the negative charges of the peptide and the positive charged polymer. At pH 4 the charge density on PACAP is too low to interact with chitosan–TGA, hence the drug was released more rapidly.

5. Conclusion

Buccal drug delivery systems are a new opportunity for the treatment of type 2 diabetes with PACAP. However, as the buccal mucosa is an effective permeability barrier, new strategies must be found to overcome this barrier. In addition to its permeation enhancing effect, chitosan–TGA shows improved mucoadhesive properties and a controlled drug release profile. Due to the addition of glutathione a 10-fold improved permeation was achieved in vitro. Based on these properties the chitosan–TGA conjugate in combination with glutathione represents a promising candidate for a safe buccal drug delivery system of PACAP.

Acknowledgements

The authors wish to thank Mr. Mayerhofer and coworkers for the supply of buccal mucosa of pigs. This work was supported by Bayer AG, 51368 Leverkusen, Germany/Europe.

References

Barrett, W.C., DeGnore, J.P., Konig, S., Fales, H.M., Keng, Y.F., Zhang, Z.Y., Yim, M.B., Chock, P.B., 1999. Regulation of PTP1B via glutathionylation of the active site cysteine 215. Biochemistry 38, 6699–6705.

Bernkop-Schnürch, A., 2000. Chitosan and its derivatives: potential excipients for peroral peptide delivery systems. Int. J. Pharm. 194, 1–13.

Bernkop-Schnürch, A., Schwarz, V., Steininger, S., 1999. Polymers with thiol groups: a new generation of mucoadhesive polymers? Pharm. Res. 16, 876–881.

Calabrese, A.T., Coley, K.C., DaPos, S.V., Swanson, D., Rao, R.H., 2002. Evaluation of prescribing practices: risk of lactic acidosis with metformin therapy. Arch. Intern. Med. 162, 434–437.

Clausen, A.E., Kast, C.E., Bernkop-Schnürch, A., 2002. The role of glutathione in the permeation enhancing effect of thiolated polymers. Pharm. Res. 19, 602–608.

Filipsson, K., Tornoe, K., Holst, J., Ahren, B., 1997. Pituitary adenylate cyclase-activating polypeptide stimulates insulin and glucagon secretion in humans. J. Clin. Endocrinol. Metab. 82, 3093–3098.

Harrower, A.D., 2000. Comparative tolerability of sulphonylureas in diabetes mellitus. Drug. Saf. 22, 313–320.

Kast, C.E., Bernkop-Schnürch, A., 2001. Thiolated polymers—thiomers: development and in vitro evaluation of chitosan–thioglycolic acid conjugates. Biomaterials 22, 2345–2352.

Ranaldi, G., Marigliano, I., Vespignani, I., Perozzi, G., Sambuy, Y., 2002. The effect of chitosan and other polycations on tight junction permeability in the human intestinal Caco-2 cell line(1). J. Nutr. Biochem. 13, 157–167.

Rathbone, M.J., Drummond, B.K., Tucker, I.G., 1994. The oral cavity as a site for systemic drug delivery. Adv. Drug Deliv. Rev. 13, 1–22.

Roldo, M., Hornof, M., Caliceti, P., Bernkop-Schnurch, A., 2004. Mucoadhesive thiolated chitosans as platforms for oral controlled drug delivery: synthesis and in vitro evaluation. Eur. J. Pharm. Biopharm. 57, 115–121.

Rosak, C., 2002. The pathophysiologic basis of efficacy and clinical experience with the new oral antidiabetic agents. J. Diabetes Complications 16, 123–132.

Schipper, N.G.M., Olsson, S., Hoogstraate, J.A., deBoer, A.G., Varum, K.M., Artursson, P., 1997. Chitosans as absorption enhancers for poorly absorbable drugs. 2. Mechanism of absorption enhancement. Pharm. Res. 14, 923–929.

Senel, S., Hincal, A.A., 2001. Drug permeation enhancement via buccal route: possibilities and limitations. J. Contr. Rel. 72, 133–144.

Senel, S., Ikinci, G., Kas, S., Yousefi-Rad, A., Sargon, M.F., Hincal, A.A., 2000. Chitosan films and hydrogels of chlorhexidine gluconate for oral mucosal *delivery*. Int. J. Pharm. 193, 197–203.

Singla, A.K., Chawala, M., 2001. Chitosan: some pharmaceutical and biological aspects—an update. J. Pharm. Pharmacol. 53, 1047–1067.

Staddon, J.M., Herrenknecht, K., Smales, C., Rubin, L.L., 1995. Evidence that tyrosine phosphorylation may increase tight junction permeability. J. Cell Sci. 108, 609–619.

Veuillez, F., Kalia, Y.N., Jacques, Y., Deshusses, J., Buri, P., 2001. Factors and strategies for improving buccal absorption of peptides. Eur. J. Pharm. Biopharm. 51, 93–109.

Wagman, A.S., Nuss, J.M., 2001. Current therapies and emerging targets for the treatment of diabetes. Curr. Pharm. Des. 7, 417–450.

Walker, G., Zarti, H., Bernkop-Schnürch, A., 2001. Thiolation of polycarbophil enhances its inhibition of intestinal brush border membrane bound aminopeptidase N. J. Pharm. Sci. 90, 1907–1914.

Wertz, P.W., Squier, C.A., 1991. Cellular and molecular basis of barrier function in oral epithelium. Crit. Rev. Ther. Drug Carrier Syst. 8, 237–269.

Xiao, Q., Giguere, J., Parisien, M., Jeng, W., St-Pierre, S.A., Brubaker, P.L., Wheeler, M.B., 2001. Biological activities of glucagon-like peptide-1 analogues in vitro and in vivo. Biochemistry 40, 2860–2869.

Yada, T., Sakurada, M., Filipsson, K., Kikuchi, M., Ahren, B., 2000. Intraperitoneal PACAP administration decreases blood glucose in GK rats, and in normal and high fat diet mice. Ann. NY Acad. Sci. 921, 259–263.