Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 348 (2008) 169–174
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Note

Design and *in vivo* evaluation of a patch delivery system for insulin based on thiolated polymers

Vjera Grabovac, Florian Föger, Andreas Bernkop-Schnürch *

Institute of Pharmacy, University of Innsbruck, Innrain 52, 6020 Innsbruck, Austria

Received 23 February 2007; received in revised form 28 June 2007; accepted 28 June 2007
Available online 15 August 2007

Abstract

Purpose: The aim of this study was to develop and evaluate a novel three-layered oral delivery system for insulin *in vivo*.
Methods: The patch system consisted of a mucoadhesive layer, a water insoluble backing layer made of ethylcellulose and an enteric coating made of Eudragit®. Drug release studies were performed in media mimicking stomach and intestinal fluids. For *in vivo* studies patch systems were administered orally to conscious non-diabetic rats. Orally administered insulin in aqueous solution was used as control. After the oral administration of the patch systems a decrease of glucose and increase of insulin blood levels were measured.
Results: The mucoadhesive layer, exhibiting a diameter of 2.5 mm and a weight of 5 mg, comprised polycarbophil-cysteine conjugate (49%), bovine insulin (26%), gluthatione (5%) and mannitol (20%). 74.8 ± 4.8% of insulin was released from the delivery system over 6 h. Six hours after administration of the patch system mean maximum decrease of blood glucose level of 31.6% of the initial value could be observed. Maximum insulin concentration in blood was 11.3 ± 6.2 ng/ml and was reached 6 h after administration. The relative bioavailability of orally administered patch system versus subcutaneous injection was 2.2%.
Conclusion: The results indicate that the patch system provides enhancement of intestinal absorption and thereby offers a promising strategy for peroral peptide delivery.

Keywords: Oral insulin; Oral delivery; Oral peptide delivery; Polymer; Intestinal absorption

1. Introduction

Oral route is the most convenient route of drug application for the patients because it offers advantages over injection such as better patient compliance, low costs and avoidance of infections and pain. However, for many peptide drugs this way of application is not feasible due to poor bioavailability. Insulin, which is used by diabetic patients, needs to be injected several times daily in order to avoid hyperglycemia. Oral administration of insulin would mean improvement of the life quality for diabetic patients. Furthermore, insulin absorbed in the small intestine would mimic the physiology of insulin, carried directly to the liver via the portal vein. Absorption into the portal vein would maintain a peripheral-portal insulin gradient that regulates insulin secretion. In its first passage through the liver, roughly 60% of the insulin is retained and metabolized, thereby reducing the incidence of peripheral hyperinsulinaemia.

However, due to its high molecular mass, charge and hydrophilicity insulin is unable to pass intestinal membranes in order to reach the blood stream in a sufficient amount. Moreover, as a peptide, insulin is susceptible to the proteolytic degradation by different gastrointestinal enzymes on the gastric and intestinal mucosa as well as by acidic environment of the stomach. Attempts to overcome enzymatic barrier and improve the bioavailability have involved, on the one hand use of liposomes (Takeuchi et al., 1996) and nanoparticles (Mathiowitz et al., 1997) as a mechanical protection and, on the other hand enzyme inhibitors, as biochemical protection. However, enzyme inhibitors have exhibited high incidence of systemic intoxications, disturbed ingestion of nutritive proteins and pancreas malfunctions (Marschütz and Bernkop-Schnürch, 2000).

The use of mucoadhesive polymers has been established as efficient drug carriers for poorly absorbed orally administered drugs. Besides not being absorbed from the intestinal tract

* Corresponding author. Tel.: +43 512 5075383; fax: +43 512 5072933.
E-mail address: andreas.bernkop@uibk.ac.at (A. Bernkop-Schnürch).

0378-5173/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2007.06.052

and guaranteeing, in that way, desirable lack of systemic toxic side effect, polymeric drug carriers offer intimate contact to the intestinal mucosa due to mucoadhesive properties. Further on, polymeric drug carrier provides prolonged residence time in the intestine, higher concentration of the drug on the mucosa, and sustained release of the drug (Bernkop-Schnürch et al., 1999). It has been reported that the mucoadhesive poly(acrylates) like Carbopol and polycarbophil are capable of enhancing the intestinal absorption of peptides by reducing the metabolic activity of both luminal and membrane bound proteolytic enzymes and by opening of the intestinal intercellular junctions (Luessen et al., 1996a,b). Thiolated polymers, or so-called thiomers, represent a new class of efficient mucoadhesive polymers with improved mucoadhesive and permeation enhancing properties (Bernkop-Schnürch, 2005). In this study thiolated polycarbophil was chosen as a polymeric drug carrier because it represents a promising combination of the enzyme activity inhibitor and efficient paracellular permeation enhancer (Bernkop-Schnürch and Thaler, 2000).

In this work we developed a multi-layered oral delivery system for insulin comprising thiolated polycarbophil as a polymeric matrix layer and water-insoluble backing layer, preventing additionally an attack of intestinal luminal enzymes. By using enteric coating the intact transport of the dosage form through the stomach could be guaranteed. Such drug delivery system may achieve increased local drug concentration and protect insulin against proteolytic degradation.

2. Experimental part

2.1. Materials

Polycarbophil was purchased from Noveon. Bovine insulin, glutathione, cysteine, ethylcellulose and Ellman's reagent (DTNB, 5,5′-dithiobis(2-nitrobenzoic acid), ethylenediaminetetraacetic acid (EDTA), dimethylsulfoxide (DMSO), ethylenediaminetetraacetic acid tripotassium salt dihydrate (K3-EDTA) and trishydroxymethylaminomethane (TRIS) were purchased from Sigma–Aldrich, Austria. Mannitol was purchased from Gatt-Koller, Austria. Eudragit L100-55 was purchased from Röhr, Germany.

2.2. Manufacturing of patches

First, polycarbophil-cysteine conjugate was synthesized as described previously (Bernkop-Schnürch and Steininger, 2000). Degree of modification was determined using Ellman's reagent (Bernkop-Schnürch and Steininger, 2000). Patches were prepared using a mixture of 49% PCP-cysteine, 26% insulin, 5% gluthatione and 20% mannitol dissolved in water and adjusted to pH 2.5 with 1 M HCl. After lyophilization 5 mg discs were compressed at constant pressure of 1.5 kN, resulting in discs of 0.5–0.8 mm height and 2.5 mm diameter. Discs were coated in two steps. Each coating step was performed by immersing the disc four times into the solution of coating material in acetone. First step included coating on the top and on the side with 5% (w/v) ethylcellulose. The uncoated side of one disc was stick

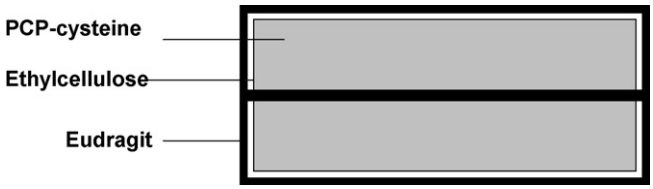

Fig. 1. Schematic presentation of the three-layered thiomer patch delivery system.

to the uncoated side of another disc using 3% (w/v) Eudragit L100-55, followed by coating of the whole dosage form with 3% (w/v) Eudragit L100-55 (Fig. 1). Thereafter, the obtained "sandwich" was damped with a tiny amount of hard fat in order to facilitate swallowing in *in vivo* experiments with rats.

2.3. In vitro release of insulin from the patches

A release profile of insulin from the patch delivery system was first evaluated in 1.2 ml, 80 mM, pH 1.2 HCl with 0.2% NaCl, mimicking an artificial gastric fluid, for 2 h. The dosage forms were placed in the orbital shaker at temperature of 37 °C provided with magnetic stirrer plate and stirred at 200 rpm. The goal of the incubation of the dosage form in the acidic medium was to demonstrate the stability of the coated formulation toward stomach acidity. After 2 h, the medium was replaced with 1.2 ml phosphate buffer containing 9 mM Na_2HPO_4; 1.6 mM KH_2PO_4; 0.14 M NaCl; 5 mM EDTA; 5 mM TRIS and 10% DMSO, adjusted to pH 7.4. DMSO has been added in order to enhance the solubility of released insulin. During the next 6 h aliquots of 120 μl were withdrawn at intervals of 30 min. Sink conditions were maintained throughout the whole experiment. An amount of released insulin was determined by HPLC (La Chrome, Hitachi, Japan) using a method described previously by our research group (Marschütz and Bernkop-Schnürch, 2000).

2.4. In vivo evaluation of the delivery system

The fate of the patch system in the gastrointestinal tract was examined as described below. Six hours after the administration, rats were sacrificed and patch systems were checked for their location in the gastrointestinal tract and shape.

The protocol for the studies on animals was approved by the Animal Ethical Committee of Vienna, Austria and adhered to the Principles of Laboratory Animal Care. For *in vivo* studies non-diabetic male Sprague–Dawley rats of body weight 264 ± 16 g were used. The rats were obtained from the Institut für Labortierkunde und Genetik, University of Vienna, Austria. The rats were fasted for 12 h prior the experiment. Before an application of insulin formulations, 90 μl of blood samples were withdrawn from the tail vein. The samples were collected in 1.5 ml tubes containing 10 μl of 15% aqueous K3-EDTA solution. These values served as reference values and time of their withdrawal was noted as zero. Thereafter, six rats were dosed orally with patch delivery system. Tablets were administered to non-anaesthetized animals by placing the tablet deep into the throat. In order to ensure the swallowing reflex, immediately after tablet administration, 200 μl of drinking water

were administered orally. To determine the relative bioavailability of the oral formulation versus subcutaneous injection, six rats received 248–280 μl subcutaneous injection containing 0.16 mg/ml insulin. Total amount of injected insulin was 0.04 mg per kg of body weight. To the third group of six rats insulin solution containing the same amount of insulin as patch system was administered orally. Oral solution containing the same amount of insulin as the patch system served as control.

During the study, dosed rats were kept in restraining cages and supplied only with drinking water. Blood samples from orally dosed rats were collected from the tail vein every 2 h during the period of 12 h, starting from the administration. In the case of rats that were given insulin subcutaneously, blood samples were withdrawn from the tail vein at the 0.25, 1, 2, 4, 6, 8 h intervals. After 12 h the rats were fed. Blood glucose level was determined using a blood glucose reader (MediSense Precision Xtra Plus, Abbott, UK).

Blood samples were centrifuged (5000 × *g* for 5 min) plasma was collected and stored at −20 °C until analysis. The amount of insulin in plasma was determined in duplicate using the ELISA for bovine insulin purchased by Mercodia, Sweden.

2.5. Pharmacokinetic analysis

c_{max} and t_{max} were determined from the pharmacokinetic profiles generated by plotting the concentration of insulin in plasma (ng/ml) versus time. The areas under the concentration time curves (AUC) were calculated according to the linear trapezoidal rule. The relative bioavailability was calculated from the dose and areas under the curves for oral versus subcutaneous administration.

3. Results

3.1. Characterization of polycarbophil-cysteine conjugate

Cysteine was bound to polycarbophil (PCP) via amide bond between the carboxylic groups of the polymer and primary amino groups of L-cysteine. PCP-cysteine conjugate exhibited 386.1 ± 23.6 μmol thiol groups per gram polymer. The synthesized conjugate was white, odorless and showed fibrous structure.

3.2. In vitro release of insulin

Diffusion studies in acidic medium showed that no insulin was released at all from the formulation within 2 h of incubation (data not shown). Drug release took place after the patch system had been placed in the medium of the pH 7.4 where Eudragit, holding the two adherent patches together, was dissolved. The release profile of insulin from patches over the period of 6 h in intestinal pH conditions is shown in Fig. 2 indicating sustained release of insulin from the polymer matrix. Over the first 3 h of the incubation at intestinal pH a zero-order release kinetic could be observed, whereby 74.6 ± 4.8% of the total insulin in the formulation was released. After 3 h the release profile reached a plateau phase.

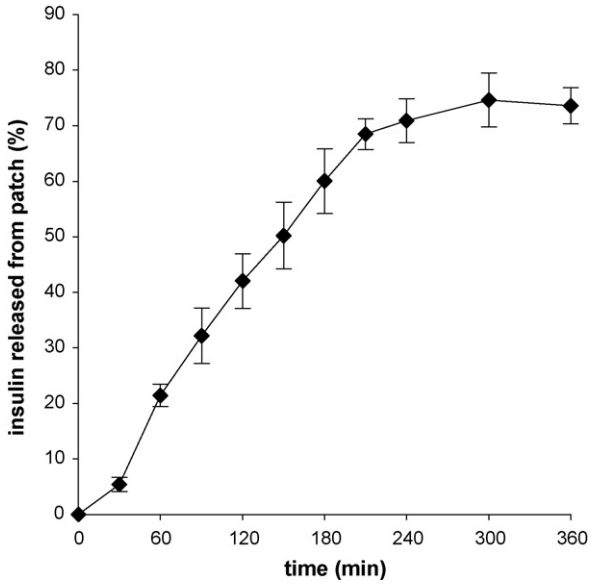

Fig. 2. Release profile of insulin from the patch delivery system. A single dosage form was incubated in phosphate buffer containing 10% DMSO, pH 7.4 at 37 °C. Cumulative corrections were made. Data represent mean ± S.D.

During the whole experiment the shape of the patches was maintained demonstrating the efficacy of the ethylcellulose coating.

3.3. In vivo studies

Fate of the patch system 6 h after the administration is shown in Fig. 3. As anticipated, after 6 h patch system was split in two patches, each adhering to the luminal side of the intestine (mid jejunum). These results indicate that a release of insulin from the patches started upon arrival into the environment of the intestinal pH.

3.3.1. Pharmacological efficacy: evaluation of the hypoglycemic effect

In order to evaluate the pharmacological efficacy of administered insulin decrease of blood glucose level, as a biological response to the administration of insulin, was determined. In Fig. 4 hypoglycemic effect induced by the oral administration of the patch in comparison to the hypoglycemic effect induced by subcutaneous injection is shown. Within first 2 h after oral administration of the patch delivery system, slight decrement of blood glucose level could be observed. This decrement can be explained by stress induced drop of blood glucose level triggered by oral application of the insulin delivery system at conscious rats, since the same decrement could be determined after oral administration of insulin solution. After 6–8 h glucose level dropped down by 31.6% of the initial value and was maintained for several hours. In contrary, oral insulin solution did not induce any significant decrease of glucose level (Fig. 4).

3.3.2. Determination of insulin in rat serum

The bioavailability of insulin delivered via thiomer patch system was evaluated by determining insulin concentration in

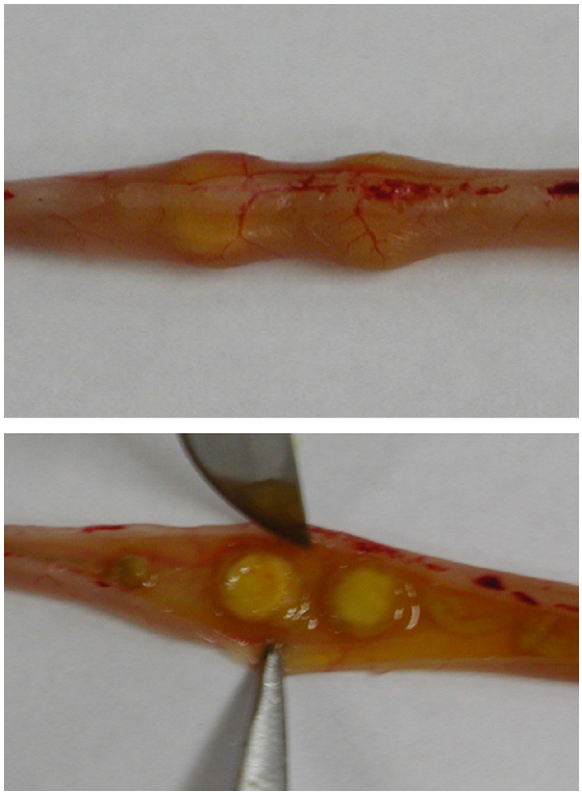

Fig. 3. Images of thiomer patch delivery system in rat mid-jejunum 6 h after oral administration.

plasma. The mean plasma insulin concentration against time profiles obtained after s.c. and oral administration of the thiomer patch system or insulin control solution is shown in Fig. 5. Subcutaneous injection showed immediate increase in insulin resulting in insulin concentration up to 32.6 ng/ml (two rats out of five). In contrary orally administered thiomer patch system induced an increment of insulin concentration up to 17.5 ng/ml (two rats out of six) 8 h after the application. Results demonstrating significant increase of insulin in serum already 4 h after administration are in good agreement with results of blood glucose measurements. In parallel, both constant plasma concentration of insulin and decreased plasma glucose level were maintained for 6 h. A relative insulin bioavailability was determined to be 2.2% versus subcutaneous injection. Main pharmacokinetic parameters are presented in Table 1.

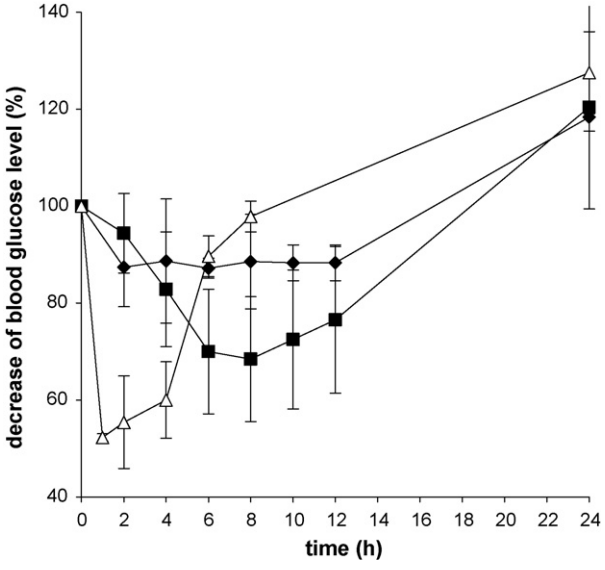

Fig. 4. Decrease of the blood glucose level (percent of an initial value) as a biological response to insulin after subcutaneous injection (△), oral administration of patch system (■) and after oral administration of insulin solution (♦). Rats were fed after 12 h. Indicated values are means ± S.D. of five rats for s.c. and six rats for oral administrations.

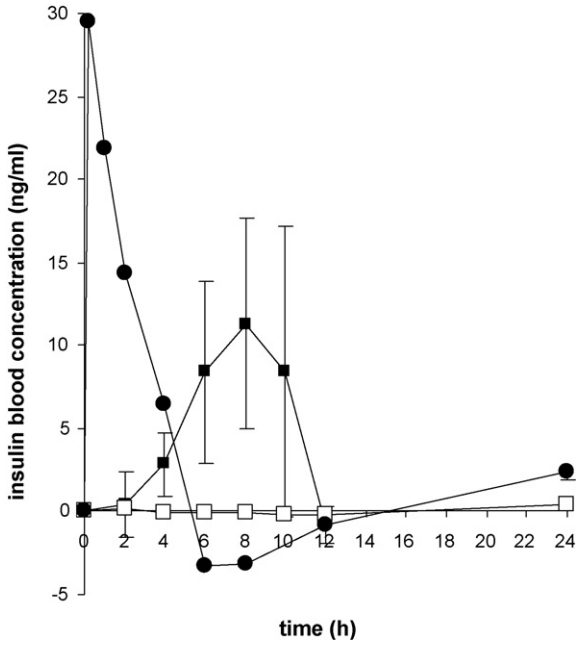

Fig. 5. Plasma insulin concentration after administration of insulin subcutaneous injection (●) insulin patch system (■) and oral insulin solution (□). Rats were fed after 12 h Indicated values represent means ± S.D. of 5–6 rats.

4. Discussion

Patches have been routinely used as transdermal delivery systems for contraceptives (Burkman, 2004), narcotic analgesic (Skaer, 2006) and nicotine (Foulds et al., 2006). Recently, patches have been also considered as promising buccal (Li et al., 1998) and oral (Eaimtrakarn et al., 2003) preparations for peptide delivery. A major objective for use of patch preparations for oral protein delivery is prevention of protein hydrolysis by luminal proteolytic enzymes and its absorption enhancement from the small intestine (Eaimtrakarn et al., 2002). In the present study we report about preparation and efficacy of oral delivery system for insulin consisting of three-layered intestinal patch. The system has been designed to both, provide prolonged residence time of the drug on the site of action and diminish a diffusion pathway of the drug from the delivery system to the absorption membrane (Shen and Mitragotri, 2002). This was achieved by using an advantage of thiolated mucoadhesive polymer matrix as a drug carrier. As previously reported, PCP-cysteine conjugate, showed excellent mucoadhesive and permeation enhancing properties in *in vitro* (Bernkop-Schnürch and Thaler, 2000) and *in vivo*

Table 1
Main pharmacokinetic parameters obtained by administration of insulin in different formulations to the rats, average weight 264 ± 16 g

Formulation	Oral patch system	Oral insulin solution	Subcutaneous injection
Insulin dose (mg/kg)	9	9	0.16
Minimum glucose level in % of initial value	70 ± 12.8	87.4 ± 8.1	52.2 ± 0.8
Time point of minimum glucose level (h)	8	–	1
c_{max} (plasma insulin ng/ml)	11.3 ± 6.2	–	29.5 ± 3.1
t_{max}	8	–	0.2
$AUC_{0->12}$/rat	62.0	0	50.6
Relative bioavailability (%)	2.2%	–	

(Schmitz et al., 2005) studies. Since PCP-cysteine is covalently bound to the mucus layer due to the thiol/disulfide exchange reactions with cysteine-rich mucin glycoprotein (Marschütz et al., 2000), an improved mucoadhesive properties can be ascribed to the thiol groups in its structure. Moreover, polymeric matrices based on polyacrylates like carbopol and polycarbophil can inhibit luminal proteases of the gastrointestinal tract and consequently, degradation of peptides (Bai et al., 1996; Luessen et al., 1997). Since luminally secreted enzymes must first penetrate the polymeric network in order to degrade the peptide polycarbophilic matrix offers, accordingly, a protection of the embedded peptide. By using a combination of pH dependent and water insoluble coating, intestinal drug targeting of intact insulin could be achieved. The pH dependent systems exploit the general acknowledged view that pH of the gastrointestinal tract increases progressively from the stomach (pH 2–3) to the small intestine (pH 6.5–7) (Leroux et al., 1995). Site specific delivery into the upper intestine can be achieved by use of Eudragit L100-55, a pH sensitive methacrylic acid copolymer with a dissolution threshold of pH 5.5. The pH-sensitivity of Eudragit L100-55 has been previously demonstrated through *in vitro* experiments mimicking pH conditions within the gastrointestinal tract (Leroux et al., 1995; De Jaeghere et al., 1998). Further on, the backing layer consisting of water insoluble ethylcellulose provides the protection against enzymatic hydrolysis (Eaimtrakarn et al., 2003) and diffusion of the drug into the intestinal lumen.

Hydrogels, such as polycarbophil, are generally highly permeable to various drug compounds, can withstand acidic environments, and can be tailored to "swell", thereby releasing entrapped molecules through their weblike surfaces (Vogelson, 2001). However, due to the relatively small diffusion area of the patch and limited water inflow, which is necessary for swelling, diffusion of insulin was problematic. In order to provide a sufficient insulin release, one single dosage was build up of two single patches stuck to each other with the layer of Eudragit L100-55. After reaching the pH 5.5 the patches were assumed to fall apart resulting in doubled diffusion area. As reported previously, by addition of mannitol, a release rate of the drug from PCP-cysteine drug carrier matrix can be significantly improved (Achleitner, 1995). Optimal quantity of added mannitol providing relatively high drug release under preservation of the patch shape was found to be 20% (w/w).

Hypoglycemic effect was taken as a measure for insulin absorbed in its pharmacologically active form. Two hours after the administration of dosage forms, a significant hypoglycemic effect could be observed. The mean maximum reduction of blood glucose appeared 6–8 h after administration, being 31.6% of the initial value and was maintained for several hours, providing evidence that insulin was, both released from patches and absorbed, in its active form. The relatively high standard deviations can be explained by usually high variations in the passage time of enteric-coated tablets from the rat stomach into the duodenum.

The use of water insoluble backing layer seems to be expedient, considering that the enzymatic activity of luminally secreted peptidase in rats is 16 times greater than the activity of the membrane-bound peptidases (Woodley, 1994). Thereby, chances for insulin degradation by proteolytic enzymes are additionally minimized.

Despite of ensured protection toward enzymatic degradation by backing layer insulin still requires a permeation enhancer, in order to be absorbed, owing to its large molecular size which is considered to be major factor limiting its diffusion across biological membranes (Hosny et al., 2006). This also explains the benefit of the PCP-cysteine over an unmodified polymer, since PCP-cysteine induces paracellular transport by opening transiently tight junctions (Bernkop-Schnürch and Thaler, 2000). GSH plays an important role in the opening of tight junctions of intestinal epithelia. PCP-Cys has the capability to reduce oxidized glutathione, prolonging the concentration of GSH at the apical membrane, resulting in significantly enhanced paracellular transport (Clausen et al., 2002).

In contrary, orally administered insulin solution showed no reduction in blood glucose level due to both, dilution of insulin with gastrointestinal fluids and degradation by proteolytic enzymes. Krauland et al. (2004) evaluated the hypoglycemic effect of orally administered insulin in non-diabetic rats by co-administering two types of enzyme inhibitors incorporated into polymeric matrix using the same amount of insulin, achieving a maximal decrement of blood glucose level for 28% of the initial value, which is in the same range like in the present study by using protective layers instead of enzyme inhibitors. Also the calculated relative bioavailability in the study by Krauland, which has been calculated to be ∼1.7% can be compared to our findings (∼2.2%).

In comparison to the delivery system for insulin based on enzyme inhibitors covalently attached to PCP-cysteine (Marschütz et al., 2000), where a reduction of plasma glucose was maintained for 80 h, an effect induced by patches has a duration of 6 h, raising to the initial concentration 12 h after administration of insulin. Taking into account the rat intestine turn over rate of up to 270 min (Lehr et al., 1991), the sustained

effect, in the study by Marschütz, cannot be attributed to the mucoadhesive properties of the delivery system but possibly to the irreversible inhibition of proteolytic enzymes. Permanent inactivation of the luminal enzymes could lead to the intestinal discomfort and digestion malfunction.

Also insulin blood levels were in good accordance with the obtained glucose blood levels. Since the insulin detection ELISA kit used shows a crossreactivity with rat insulin it was necessary to subtract the initial values. As shown in Fig. 5, insulin blood levels of the rats treated with oral insulin solution did not change over the 12 h of experiment indicating the efficacy of the system. One may argue that the dose of administered insulin is too high, but since the ability of non-diabetic rats to compensate the reduction of glucose level is higher than in non-diabetic rats, it was necessary to apply such high dosages of insulin in order to obtain the valid results.

Using enteric coating material and water insoluble backing layer it was possible to shield insulin from stomach acidity and proteolytic enzymes and target the site of the drug delivery. The polymeric matrix comprising of mucoadhesive thiolated polymer enabled a sustained release of the insulin providing a steep concentration gradient on the intestinal membrane over the period of 6 h.

References

Achleitner, B., 1995. bioadhäsive Arzneiformulierungen. freisetzung, stabilität und dotierung mit fimbrien, Diplomathesis University of Vienna.

Bai, J.P.F., Chang, L.L., Guo, J.H., 1996. Effects of polyacrylic polymers on the degradation of insulin and peptide drugs by chymotrypsin and trypsin. J. Pharm. Pharmacol. 48, 17–21.

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

Bernkop-Schnürch, A., Steininger, S., 2000. Synthesis and characterisation of mucoadhesive thiolated polymers. Int. J. Pharm. 194, 239–247.

Bernkop-Schnürch, A., Thaler, S., 2000. Polycarbophil-cysteine conjugates as platforms for oral (poly)peptide delivery systems. J. Pharm. Sci. 89, 901–909.

Bernkop-Schnürch, A., 2005. Thiomers: a new generation of mucoadhesive polymers. Adv. Drug. Deliv. Rev. 57, 1569–1582.

Burkman, R.T., 2004. Transdermal contraceptive systems. Am. J. Obstet. Gynecol. 190, 49–53.

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.

De Jaeghere, F., Allémann, E., Kubel, F., Galli, B., Doelker, E., Gurny, R., 1998. pH-Sensitive microparticles as oral delivery systems for antiinfective agents of poor water solubility. In: Proceedings of the 2nd World Meeting APGI/APV 2, pp. 559–560.

Eaimtrakarn, S., Itoh, Y., Kishimoto, J., Yoshikawa, Y., Shibata, N., Murakami, M., Takada, K., 2002. Gastrointestinal mucoadhesive patch system (GI-MAPS) for oral administration of G-CSF, a model protein. Biomaterials 23, 145–152.

Eaimtrakarn, S., Prasad, Y.V.R., Puthli, S.P., Yoshikawa, Y., Shibata, N., Takada, K., 2003. Possibility of a patch system as a new oral delivery system. Int. J. Pharm. 250, 111–117.

Foulds, J., Steinberg, M.B., Williams, J.M., Ziedonis, D.M., 2006. Developments in pharmacotherapy for tobacco dependence: past, present and future. Drug Alcohol Rev. 25, 59–71.

Hosny, E.A., Ghilzai, N.M.K., Elmaza, M.M., 2006. Promotion of oral insulin absorption in diabetic rabbits using pH-dependent coated capsules containing sodium cholate. Pharm. Acta Helvetiae 72, 203–207.

Krauland, A.H., Guggi, D., Bernkop-Schnürch, A., 2004. Oral insulin delivery: the potential of thiolated chitosan-insulin tablets on non-diabetic rats. J. Control. Rel. 95, 547–555.

Lehr, C.-M., Poelma, F.G.J., Junginger, H.E., Tukker, J.J., 1991. An estimate of turnover time of intestinal mucus gel layer in the rat in situ loop. Int. J. Pharm. 70, 235–240.

Leroux, J.C., Cozens, R., Roesel, J.L., Galli, B., Kubel, F., Doelker, E., Gurny, R., 1995. Pharmacokinetics of a novel HIV-1 protease inhibitor incorporated into biodegradable or enteric nanoparticles following intravenous and oral administration to mice. J. Pharm. Sci. 84, 1387–1391.

Li, C., Bhatt, P.P., Johnston, T.P., 1998. Evaluation of a mucoadhesive buccal patch for delivery of peptides: in vitro screening of bioadhesion. Drug Dev. Ind. Pharm. 24, 919–926.

Luessen, H.L., Bohner, V., Perard, D., Langguth, P., Verhoef, J.C., de Boer, A.G., Merkle, H.P., Junginger, H.E., 1996a. Mucoadhesive polymers in peroral peptide drug delivery. V. Effect of poly(acrylates) on the enzymatic degradation of peptide drugs by intestinal brush border membrane vesicles. Int. J. Pharm. 141, 39–52.

Luessen, H.L., de Leeuw, B.J., Perard, D., Lehr, C.-M., de Boer, A.G., Verhoef, J.C., Junginger, H.E., 1996b. Mucoadhesive polymers in peroral peptide drug delivery. I. Influence of mucoadhesive excipients on the proteolytic activity of intestinal enzymes. Eur. J. Pharm. Sci. 4, 117–128.

Luessen, H.L., Rentel, C.O., Kotzé, A.F., Lehr, C.-M., deBoer, A.G., Verhoef, J.C., Junginger, H.E., 1997. Mucoadhesive polymers in peroral peptide drug delivery. IV. Polycarbophil and chitosan are potent enhancers of peptide transport across intestinal mucosae in vitro. J. Control. Rel. 45, 15–23.

Marschütz, M.K., Bernkop-Schnürch, A., 2000. Oral peptide drug delivery: polymer-inhibitor conjugates protecting insulin from enzymatic degradation in vitro. Biomaterials 21, 1499–1507.

Marschütz, M.K., Caliceti, P., Bernkop-Schnürch, A., 2000. Design and in vivo evaluation of an oral delivery system for insulin. Pharm. Res. 17, 1468–1474.

Mathiowitz, E., Jacob, J.S., Jong, Y.S., Carino, G.P., Chickering, D.E., Chaturvedi, P., Santos, C.A., Vijayaraghavan, K., Montgomery, S., Bassett, M., Morrell, C., 1997. Biologically erodable microspheres as potential oral drug delivery systems. Nature 386, 410–414.

Schmitz, T., Leitner, V.M., Bernkop-Schnürch, A., 2005. Oral heparin delivery: design and in vivo evaluation of a stomach-targeted mucoadhesive delivery system. J. Pharm. Sci. 94, 966–973.

Shen, Z.C., Mitragotri, S., 2002. Intestinal patches for oral drug delivery. Pharm. Res. 19, 391–395.

Skaer, T.L., 2006. Transdermal opioids for cancer pain. Health Qual. Life Outcomes 4, 24.

Takeuchi, H., Yamamoto, H., Niwa, T., Hino, T., Kawashima, Y., 1996. Enteral absorption of insulin in rats from mucoadhesive chitosan-coated liposomes. Pharm. Res. 13, 896–901.

Vogelson, C.T., 2001. Innovative pharmaceutical treatments require innovative methods of administrations. Modern Drug Discov. 4, 49–50.

Woodley, J.F., 1994. Enzymatic barriers for GI peptide and protein delivery. Crit. Rev. Ther. Drug Carrier Syst. 11, 61–95.