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Intestinal absorption of human insulin in pigs using delivery systems based on superporous hydrogel polymers

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Abstract

In this in vivo study, novel delivery systems based on superporous hydrogel (SPH) and SPH composite (SPHC) polymers were used to improve the intestinal absorption of insulin in healthy pigs. Six female pigs of approximately 35 kg body weight were used. A cannula was inserted into the jugular vein for blood sampling and a silicone fistula in the duodenum for administration of gelatin capsules containing the delivery systems or insulin solutions. The delivery systems consisted of two components, (1) conveyor system made of SPH and SPHC; (2) core containing insulin. The core was inserted either into the conveyor system (core inside, c.i.) or attached to the surface of conveyor system (core outside, c.o.). The following intestinal formulations were investigated: c.i., c.o. and intraduodenal (i.d.) administration of insulin solutions. Subcutaneous (s.c.) injection of insulin was also investigated for reasons of comparison. Blood samples were taken and analyzed for insulin and glucose concentrations. Relative bioavalibility values of 1.3 ± 0.4 and $1.9 \pm 0.7\%$ were achieved for c.o. and c.i. administrations, respectively. The bioavalibility for i.d. administration of insulin solution was $0.5 \pm 0.2\%$. These results indicate that the absorption of insulin was slightly increased using SPH/SPHC-based delivery systems. Furthermore, a large variability was observed, probably due to physiological and metabolic changes during the experiments. Blood glucose levels were slightly decreased after the c.o. and c.i administrations, whereas these levels did not decrease after i.d. administration of insulin solutions. In conclusion, SPH/SPHC-based delivery systems are able to enhance the intestinal absorption of insulin and are, therefore, considered as promising systems for peroral peptide drug delivery. However, insulin delivery from these delivery systems under in vivo have to be improved.

Keywords: Superporous hydrogels (SPH); SPH composite (SPHC); Insulin; Intestinal peptide absorption; Pigs

1. Introduction

Until now the most trustful way of insulin delivery for treatment of diabetes mellitus with respect to the appropriate dose is the use of

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injectable dosage forms, including infusion pumps (Irsigler et al., 1981) and devices like jet injectors (Houtzagers et al., 1988) and injection pens (Walters et al., 1985). Nevertheless, disadvantages such as low patient compliance, high costs of preparation and possible infection and pain during injection still remain for these injectables. An oral dosage form of insulin is most desirable because of its high patient compliance and preparation at a lower price (Aboubakar et al., 2000; Choudhari et al., 1994; Tozaki et al., 2001). In addition, following oral administration insulin would be directly transported from the gut into liver, thereby avoiding the peripheral hyperinsulinemic side effects (Saffran et al., 1997; Ziv et al., 1994). Peroral administration of insulin has been studied in different experimental models such as rats (Kisel et al., 2001), rabbits (Hosny et al., 1997) and dogs (Ziv et al., 1994). However, there has been little success in achieving acceptable oral bioavailability for insulin in these animal models, due to factors like enzymatic breakdown in the gastrointestinal tract, low absorption through the intestinal epithelium and the lack of possibility to increase the retention time of the delivery system at specific site(s) of absorption during which insulin can be released and absorbed (Aboubakar et al., 2000; Fix, 1996). Various delivery systems have been developed to improve the peroral administration of insulin such as microtablets (Ziv et al., 1994), pellets (Tozaki et al., 2001), nanocapsules (Aboubakar et al., 2000) and liposomes (Kisel et al., 2001). Each of these reported delivery systems managed to increase plasma insulin levels by different mechanisms of action, although not sufficiently for the therapeutic purposes.

In the present study, novel delivery systems based on superporous hydrogel (SPH) and SPH composite (SPHC) polymers, as recently developed in our laboratory (Dorkoosh et al., 2001), were evaluated on intestinal insulin absorption in healthy pigs. SPH and SPHC polymers are fast swelling hydrogels with superabsorbent properties (Chen and Park, 2000). SPH is swelling faster than SPHC, but it is mechanically less stable than SPHC. These polymers are able to increase the retention time of the delivery system at specific site(s) of drug absorption in the intestine by their fast swelling properties and subsequent mechanical attachment to the gut wall (Dorkoosh et al., 2000). The advantage of SPH/SPHC-based delivery systems over the above mentioned systems for peroral peptide drug delivery is their mechanism of action by mechanical interaction, leading to an increase of retention time and intimate contact at the intestinal epithelium (Dorkoosh et al., 2001, 2002a). The purpose of the present investigation was to evaluate the capability of these developed delivery systems on the intestinal absorption of insulin as a relatively large hydrophilic peptide drug.

2. Materials and methods

2.1. Materials

Human insulin was a generous gift from Aventis Research and Technologies (Frankfurt, Germany). PEG 6000 (polyethylene glycol) was obtained from Fluka (Zwijndrecht, The Netherlands). Superporous hydrogel (SPH) and SPHC were synthesized in our laboratory (Dorkoosh et al., 2000). Gelatin capsules were kindly donated by Capsugel® (Colmar, France). Narketan® (ketamine) was purchased from Chassot (Vught, The Netherlands). Stresnil® (azaperon) was from Jansen–Cilag (Tilburg, The Netherlands). Vials containing Li–Heparin (Monovette®) for blood sample collection were purchased from Sarstedt (Etten-Leur, The Netherlands). All other compounds were of analytical grade.

2.2. Methods

2.2.1. Preparation of SPH- and SPHC-based delivery systems

Two types of SPH- and SPHC-based delivery systems were used (Dorkoosh et al., 2001):

1) Core inside the delivery system (c.i.) which consisted of two components, (1) conveyor system made of SPH and SPHC; (2) core containing insulin (Fig. 1A). In order to prepare the conveyor system, firstly SPH and SPHC polymers were synthesized as described

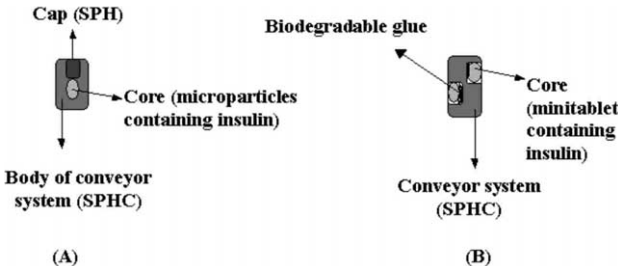

Fig. 1. Schematic figures of SPH- and SPHC-based delivery systems; (A) c.i. delivery system, (B) c.o. (attached to surface of) delivery system.

previously (Dorkoosh et al., 2000), and then a hole was made in SPHC which was used as the body. Secondly, the core component consisting of microparticles with insulin was prepared. To make these microparticles 72 mg PEG 6000 was melted. Then 28 mg insulin (activity: 25 IU/mg) was completely dispersed in the melted PEG 6000 while it was cooling down (Dorkoosh et al., 2002b). The cooled mass was crushed using a mortar and sieved through sieve mesh size 400 μm. Microparticles smaller than 400 μm were used as the core formulation. These microparticles were filled in the hole inside the SPHC, and the hole was closed with a piece of SPH as a cap. The reason for using SPH as a cap is that the swelling ratio of SPH is higher than SPHC and that the cap is ejected, allowing a burst release of the peptide drug.

2) Core attached to the surface of the delivery system (c.o.), which consisted also of two components, (1) the conveyor system which is made of SPHC; (2) the core formulated as minitablets which contains insulin (Fig. 1B). Initially, two holes were made at opposing sides of the conveyor system. For preparation of the core, 86 mg PEG 6000 were melted and during cooling down 14 mg insulin were completely dispersed in PEG (Dorkoosh et al., 2002b). The cooled mass was crushed in a mortar, sieved through sieve mesh size 400 μm and the microparticles smaller than 400 μm were mixed with Mg-stearate (1%) and compressed to minitablets with a diameter of 4 mm. Two minitablets were attached to the holes made earlier at opposing sides of conveyor system using a biodegradable glue (Histoacryl®).
3) Both systems (c.i. and c.o.) were placed in a gelatin capsule (size 000) and used for intraduodenal administration.

2.2.2. Animals and surgery

This study was performed in healthy pigs according to the approved protocol of the Ethical Committee for animal experimentation (Veterinary Faculty of Utrecht University, Utrecht, The Netherlands). Female pigs of about 35 kg body weight were used. Two weeks in advance of the start of the experiments, the pigs were housed in the animal facilities of the Central Laboratory Animal Institute (Utrecht University, Utrecht, The Netherlands) for environmental adaptation. One week before starting the insulin absorption studies, a silicone fistula (T-shaped, 2 cm o.d. and 1.5 cm i.d.) was inserted into the duodenum and a jugular vein was cannulated using the surgical procedure reported previously (Thanou et al., 2001).

The animals were fasted the night before each experiment (water access ad libitum), and had access to food 4 h after the start of the experiments.

2.2.3. Administration of insulin formulations

Before each administration, the animals were anaesthetized by subcutaneous injection of a 1:1 mixture of azaperon/ketamine (each 0.1 ml/kg body weight) to facilitate the administrations. The duration of this anesthesia was between 20 and 30 min. Capsules were administered using a custom-made flexible plunger applicator via the duodenal fistula, and insulin solutions using a 20 ml syringe attached to a flexible tube via the fistula. Subcutaneous administrations were performed in the lateral skin of the hind limb.

The following formulations were applied to the pigs according to a randomized cross-over setup (Table 1): subcutaneous injection of insulin (s.c.); intraduodenal administration of insulin solution (i.d.); gelatin capsules containing delivery systems with core outside the conveyor (c.o.); gelatin capsules containing delivery systems with core inside the conveyor (c.i.); gelatin capsules contain-

Table 1
Administration of different insulin formulations

Type of administration	Insulin dose (IU/kg)	Amount of administration
S.c. (subcutaneous)	0.2	5 ml containing 7 IU insulin
I.d. (intraduodenal)/ solution	20	20 ml containing 700 IU insulin
C.o./i.d.	20	Dosage form with 700 IU insulin
C.i./i.d.	20	Dosage form with 700 IU insulin
Only SPHC polymer/ i.d.	–	Capsule with only polymer
Control s.c.	–	5 ml physiological saline

ing only 600 mg SPHC polymer (as i.d. negative control); subcutaneous injection of physiological saline (as s.c. negative control).

For s.c. injection, 0.28 mg insulin was added to 5 ml physiological saline (0.9% NaCl). The pH was firstly lowered to 3 with 0.2 M HCl to dissolve insulin completely and then increased to pH 7.2 with 0.2 M NaOH. For i.d. administration of insulin solutions, 28 mg insulin was dissolved in 20 ml physiological saline at pH 7.2 as described for the s.c. solutions. The formulations were administered to the animals at 48 h interval between administrations to ensure complete wash-out of the peptide drug. Blood samples of 4 ml were collected using blood-heparinized collecting syringes and substituted by 4 ml sterile pyrogen-free physiological saline. Heparinized physiological saline (2 ml; 25 U/ml) was administered to fill the dead volume of the cannula to avoid blood clotting. Blood samples were withdrawn at the following time intervals: −5, 15, 30, 60, 90, 120, 150, 180, 240 and 300 min. They were kept on ice and then centrifuged for 10 min at 3000 rpm and 4 °C. The plasma was separated and kept at −20 °C until analysis.

At the end of all experiments the animals were euthanized by an overdose of pentobarbital and the GI tract was inspected macroscopically for possible damage.

2.2.4. Measurement of blood glucose and plasma insulin levels

Immediately after blood sampling, the glucose concentration was measured using a blood glucose meter (Accutrend Sensor, Roche Diagnostics, Almere, The Netherlands) according to the manufacturer's protocol.

Plasma samples were analyzed for the amount of insulin by a commercially available radioimmunoassay (Pharmacia & Upjohn, Uppsala, Sweden) with a detection limit of 2 μU/ml. The analysis was performed according to the manufacturer's protocol.

2.2.5. Pharmacokinetic analysis of data

Pharmacokinetic parameters, including total area under the plasma concentration–time curve (AUC) and mean insulin concentration after each administration (C_{ins}; average of absorbed insulin at each time interval), were obtained directly from the plasma insulin concentrations. The AUCs for each administration were calculated by the linear trapezoidal rule (Gibaldi and Perrier, 1975). Relative bioavailability values after intraduodenal administration of insulin were calculated according to:

$$F_R = \frac{AUC_{i.d.} \times D_{s.c.}}{AUC_{s.c.} \times D_{i.d.}} \times 100\%$$

in which F_R is the relative bioavailability and D the administration dose.

The obtained data were evaluated for statistically significant differences by one-way analysis of variance (ANOVA) at $P < 0.05$.

3. Results and discussion

The mean plasma insulin concentration versus time profiles obtained after s.c. and intestinal administration of the different dosage forms are presented in Fig. 2. S.c. injection showed a rapid increase in insulin concentrations up to 55±24 μU/ml within 30 min post-administration. In case of intraduodenal administration of the c.o. and c.i. delivery systems, the plasma insulin concentrations increased gradually up to 27±10 and 35±14 μU/

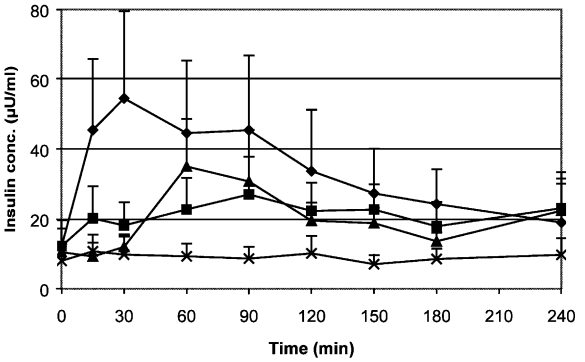

Fig. 2. Plasma concentrations (mean ± S.E.M.; $n = 6$) of insulin after administration of various formulations; (◆) s.c. (subcutaneous); (■) c.o. (intraduodenal application of delivery system with core outside); (▲) c.i. (intraduodenal application of delivery system with core inside); (×) i.d. (intraduodenal application of insulin solution).

ml within 90 and 60 min, respectively. This indicates that the c.o. and c.i. delivery systems are able to enhance the intestinal absorption of insulin compared with intraduodenal administration of insulin solutions, where no significant increases in plasma insulin concentrations were observed. From Fig. 2, it is also clear that the insulin concentrations varied considerably, probably due to various factors such as environmental condition, other hormonal cycles like adrenaline secretion from hyperkidney gland, anaesthesia of the animals, various targeting places of the delivery systems in the intestinal tract and stress during blood sampling (Damgé et al., 1990; Tozaki et al., 1997). This variability was also observed in the endogenous insulin concentrations, since the plasma levels of insulin at 5 min before the start of the experiments were also fluctuating (Table 2). These large variations just before administration can be additionally explained by hormonal changes due to environmental and/or metabolism factors during the fasting period.

Fig. 3 shows the blood glucose levels after administration of the various insulin formulations. These levels decreased very rapidly down to 40% of their initial values at 60 min after s.c. insulin injection; these low levels were maintained for about 2 h and then increased slowly. This decrease in blood glucose in each individual pig revealed

Table 2
Plasma insulin levels at 5 min before administration of the formulations

Administration	Insulin (µU/ml)	n
S.c. (insulin solution)	12.1 ± 5.1	6
S.c. (physiological saline)	6.8 ± 2.4	3
I.d. (insulin solution)	7.9 ± 4.9	6
I.d. (only SPHC polymer)	5.2 ± 0.6	3
C.o./i.d.	12.2 ± 7.4	6
C.i./ i.d.	10.4 ± 3.0	6

Results are expressed as mean ± S.E.M. for the mentioned number of experiments (n).

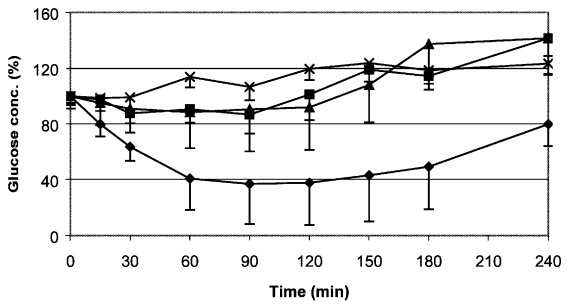

Fig. 3. Blood levels (mean ± S.E.M.; $n = 6$) of glucose after administration of various formulations; (◆) s.c. (subcutaneous); (■) c.o. (intraduodenal application of delivery system with core outside); (▲) c.i. (intraduodenal application of delivery system with core inside); (×) i.d. (intraduodenal application of insulin solution).

that, regardless of the extent of increase in plasma insulin, the amount of blood glucose was decreased to almost the same low concentrations. After intraduodenal administration of insulin solutions, the glucose levels showed a minor increase, probably due to metabolic changes and endogenous secretion of glucagon by the applied stress during blood sampling (Havel and Taborsky, 1989; Johnson et al., 1995). However, the glucose levels showed low decreases up to 150 min after intestinal administration of the c.o. and c.i. delivery systems compared with intestinal administration of insulin solutions. S.c. injection of physiological saline and intraduodenal administration of capsules containing only SPHC polymer as negative controls did not result in a significant

increase of plasma insulin levels and decrease of blood glucose concentrations (data not shown).

After feeding the animals at 4 h after the start of experiments, the blood glucose and plasma insulin levels increased rapidly within 1 h. (Fig. 4A and B). This indicates that even by changing one factor (e.g. feeding the animals), the endogenous plasma insulin levels can vary considerably. This variation was not similar for each individual pig and showed that plasma insulin is quite sensitive to any internal changes and dependent on the physiology and metabolic activity of the animals.

The pharmacokinetic parameters after intraduodenal administration of the insulin formulations are presented in Table 3. Mean insulin concentrations in plasma (C_{ins}) after c.o. and c.i. administrations were 14.1 and 19.6 μU/ml, respectively. These values were significantly higher than C_{ins} after i.d. administration of insulin solutions (8.7 μU/ml). This demonstrates that SPH/SPHC-based

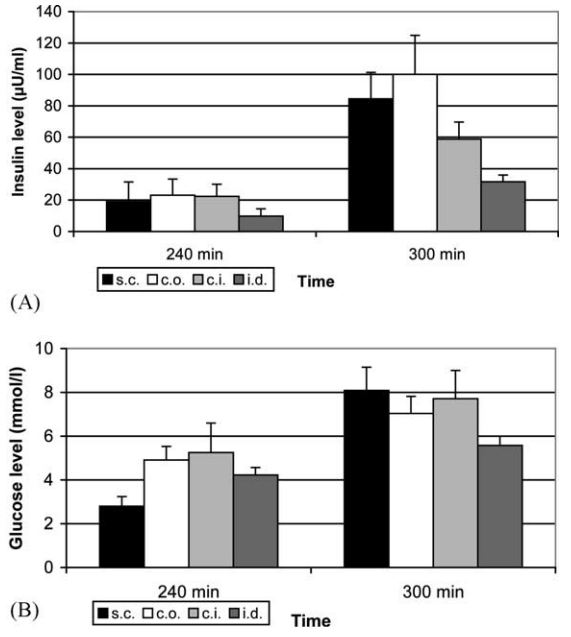

Fig. 4. Plasma insulin (A) and blood glucose (B) concentrations after feeding the animals at 240 min after start of the experiments. Data are expressed as mean ± S.E.M. of six pigs for different administrations; (■) s.c. (subcutaneous); (□) c.o. (intraduodenal application of delivery system with core outside); (▨) c.i. (intraduodenal application of delivery system with core inside); (▩) i.d. (intraduodenal application of insulin solution).

delivery systems, both with c.o. and c.i., are able to increase the intestinal absorption of insulin.

The efficacy of the present systems can be explained as follows: When the gelatin capsules containing the SPH- and SPHC-based delivery systems are placed in the gut, the capsules will dissolve and the SPHC conveyor systems will swell very quickly. Subsequently, the delivery system attaches mechanically to the absorption site in the intestine (Dorkoosh et al., 2001, 2002a). This swelling of the polymers and mechanical attachment to the gut wall will enhance the absorption of insulin, due to increased residence time of the delivery system in the gut and eventually opening of the tight junctions by mechanical pressure and water influx from the interstitial spaces (Dorkoosh et al., 2002a). This water influx causes the intestinal cells to shrink. As a consequence, the homeostatic pressure of the cells will be changed. In order to compensate for this alteration, the tight junctions may be opened to allow water molecules (together with insulin molecules) to be taken up and to maintain the homeostasis of the intestinal cells, thereby also enhancing intestinal insulin absorption (Dorkoosh et al., 2002c). The present SPH/SPHC-based delivery systems appeared to improve the intestinal insulin absorption via a new mechanism, i.e. mechanical fixation, compared with other developed delivery systems such as liposomes which enhance insulin absorption via encapsulation in phospholipid bilayers (Kisel et al., 2001), nanocapsules which protect insulin degradation by covering insulin in poly(isobutylcyanoacrylate) (Aboubakar et al., 2000) and nanoparticles which increase insulin absorption by using hydrolysable porous spheres as drug targeting vehicles (Couvreur et al., 1980).

As is evident from Table 3, the relative insulin bioavailability (F_R) values after c.o. and c.i. administrations were 1.3 ± 0.4 and $1.9 \pm 0.7\%$, respectively, showing a 2.6- and 3.8-fold enhancement in comparison to F_R values ($0.5 \pm 0.2\%$) after intraduodenal administration of insulin solutions. These enhancement factors are expected to be even higher if pigs with experimentally induced diabetes mellitus would have been used. Since it was obvious from the present study that plasma insulin levels are easily influenced by internal or external

Table 3
Pharmacokinetic parameters of insulin after intraduodenal administration in pigs

Pig number	C.o.			C.i.			I.d.		
	C_{ins} (μU/ml)	AUC (μU/ml min)	F_R (%)	C_{ins} (μU/ml)	AUC (μU/ml min)	F_R (%)	C_{ins} (μU/ml)	AUC (μU/ml min)	F_R (%)
1	22.7	5219	2.6	29.6	7979	4.1	8.4	1925	0.9
2	9.9	2568	2.4	17.4	4461	4.2	16.6	432	0.4
3	15.3	4131	0.3	36.4	9295	0.7	2.2	2933	0.2
4	18.6	4739	1.2	13.8	3590	0.9	19.9	4866	1.2
5	10.0	2578	0.8	9.5	2261	0.7	4.7	1297	0.4
6	8.1	2046	0.3	11.9	3216	0.5	0.4	97	0.02
Mean	14.1[a]	3547	1.3	19.6[a]	5134	1.9	8.7[a]	1925	0.5
S.E.M.	2.4	539	0.4	4.4	1157	0.7	3.2	722	0.2

C.o., core outside delivery system; c.i., core inside delivery system; i.d., intraduodenal administration of insulin solution. C_{ins}, mean plasma insulin concentration; AUC, area under curve; F_R, relative bioavailability.

[a] C.o. and c.i. are significantly different from i.d. ($P < 0.05$).

factors, it is recommended to use diabetic animals in order to avoid any interference of endogenously secreted insulin and to determine the absorption of intraduodenally administered insulin more accurately. Furthermore, improvement of the delivery drugs systems and using a non-endogenous peptide will improve the peroral absorption of peptide drugs as octreotide (Michael et al., 2000; Dorkoosh et al., 2002d).

4. Conclusion

This study showed the capability of SPH/SPHC-based delivery systems to enhance the intestinal absorption of insulin in healthy pigs. Although the plasma insulin levels increased after intraduodenal administration of these delivery systems, only a slight decrease in blood glucose levels was observed. Moreover, the interindividual variation was very high, probably due to other hormonal secretions or metabolic activity of the animals. Therefore, it is recommended to use diabetic pigs in order to avoid the interference of endogenous insulin with exogenously administered insulin.

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