JPP Journal of Pharmacy and Pharmacology

JPP 2004, 56: 1101–1107
© 2004 The Authors
Received April 20, 2004
Accepted June 15, 2004
DOI 10.1211/0022357044175
ISSN 0022-3573

Oil-based formulations for oral delivery of insulin

Chun-Lei Li and Ying-Jie Deng

Abstract

Several oil-based solution formulations of insulin were prepared, in which insulin was solubilized in the form of anhydrous reverse micelles. The preparation process involved micellar dissolution of insulin followed by freeze drying, then reconstitution of lyophilized product with an oil phase. These formulations were stable at room temperature for up to 12 months. No significant changes in the appearance were observed and no degradation products of insulin were detected during the course of the stability study. The efficacy of these formulations was evaluated in-vivo using diabetic Wistar rat as an animal model and then a specific formulation was chosen for further study in non-diabetic New Zealand rabbits. It was found that the efficacy of insulin oil solution was dose dependent and insulin oil solution had the same efficacy as insulin emulsion with the same formulation composition. If ethylenediaminetetraacetic acid (EDTA) was pre-delivered 40 min before the delivery of insulin oil solution, the hypoglycaemic effect of insulin oil solution was greatly enhanced, with an AUC (% glucose reduced) value increase from 28.5 ± 14.7 to 167.1 ± 72.3. The improvement of oral absorption induced by predelivery of EDTA might be attributed to enzyme inhibition, reduced gut mobility and the opening of paracellular routes.

Introduction

Insulin is a 51-amino-acid polypeptide widely used for the treatment of diabetes mellitus. More than 30 million people around the world suffer from insulin-dependent diabetes mellitus and require parenteral injections of insulin. The most common form of this therapy is twice-daily subcutaneous injections. This type of treatment is painful and inconvenient, resulting in poor patient compliance (Pillai & Panchagnula 2001). Therefore, other routes of administration have been developed, such as nasal, rectal, pulmonary and ocular routes (Trehan & Ali 1998). Among the alternative routes for insulin administration, the oral route is the most convenient and physiological because insulin undergoes a first hepatic bypass, thus ensuring a primary effect by inhibiting hepatic glucose output (Cournarie et al 2002).

However, several problems must be overcome before insulin can be efficiently orally administered, such as proteolytic breakdown in the gastrointestinal tract (Bernkop-Schnurch 1998) and absorption through the intestinal epithelium (Fix 1996). To bypass these two main barriers, many different strategies have been attempted. To date, permeation enhancers (Fix 1996), protease inhibitors (Bernkop-Schnurch 1998), enteric coating (Hosny et al 1997, 2002; Tozaki et al 2001), liposomes (Muramatsu et al 1996; Kisel et al 2001), nanocapsules (Watnasirichaikul et al 2002), pH-responsive gels (Lowman et al 1999) and microspheres (Damge et al 1997; Radwan 2001; Shimoda et al 2001) have been applied with varying degrees of success.

Recently, a novel approach has been developed for solubilizing peptides (or proteins) in hydrophobic solvents (e.g. oils) (New & Kirby 1997). The solubilization of hydrophilic peptides (or proteins) in oil phase rather than aqueous media can mask the hydrophilic surfaces of peptides, and might assist dissolved peptides in crossing cellular membranes and other biological barriers, such as the skin and the gastrointestinal mucosa. To test this hypothesis, a series of oil-based insulin formulations were prepared and their efficacy following oral administration was tested using diabetic rat and non-diabetic rabbit as animal models.

Department of Pharmaceutical Sciences, Shenyang Pharmaceutical University, No.103, Wenhua Road, Shenyang City, Liaoning Province, 110016, P. R. China

Chun-Lei Li, Ying-Jie Deng

Correspondence: C.-L. Li, Box 52, Shenyang Pharmaceutical University, Shenyang City, Liaoning Province, 110016, P. R. China.
E-mail: lcllib@sina.hotmail.com

Acknowledgement: We are grateful to Ms Dan Liu and Mr En-Sheng Tian for their assistance in the experimental work. The reviewers are acknowledged for their good suggestions and for the supply of a stability-specific HPLC method for insulin analysis (Method 2).

Materials and Methods

Materials

Three different kinds of soybean phospholipids were used in this study, which were kind gifts from Degussa (Freising, Germany), including Epikuron HL50 (phospholipid blend, enzymatically hydrolysed, highly enriched with lyso-phospholipids), Epikuron 200 (phospholipid fraction, minimum 92% PC), and Epikuron 200SH (phospholipid fraction, minimum 95% PC, hydrogenated). Sodium ursodeoxycholate was generously provided by Tianjin Pacific Pharmaceutical Co. Ltd (Tianjin, China). Medium-chain partial glycerides (trademark: Akoline MCM) was obtained from Karlshamns AB (Karlshamn, Sweden). Polysorbate 80 (trademark: Crillet 4) was a product of Croda (Singapore). Insulin (27.6 IU mg^{-1}) was purchased from Xuzhou Wanbang Biochemical Company (Xuzhou, China). All other chemicals were of analytical reagent grade.

Oil-based formulations of insulin

Three different oil-based formulations were prepared in this study. The main difference between these three formulations was that different phospholipids were used. Briefly, 30 mg phospholipids were added to 1.0 mL purified water under magnetic stirring to form the suspension of liposomes. Sodium ursodeoxycholate (70 mg) and 2 mg sodium citrate were dissolved in 1.0 mL purified water. The bile salt solution was mixed with the suspension of liposomes in a 1:1 volume ratio. The resulting mixed micelle solution was filtered through 220 nm pores to remove insoluble substances. Then 5 mg insulin was dissolved in 1.0 mL mixed micelle solution. This insulin-containing solution was filled into the 10-mL freeze-drying vials with a fill volume of 1.0 mL and lyophilized in a laboratory freeze-drier (FD-1; Beijing Bioking Technology Company, Beijing, China). The freeze-drying process was as follows: freezing at −40°C for 8 h; primary drying at −40°C for 48 h; secondary drying at 25°C for 10 h. The chamber pressure was maintained at 20 pascals during the drying process. After lyophilization, 1 mL oil phase was added to the freeze-drying vial, resulting in the formation of an optically clear oil solution. The oil phase was composed of Akoline MCM and Crillet 4 (9:1, w/w). Here a small fraction of Crillet 4 was used to increase the self-emulsification properties of oil solutions.

Formulation 1 contained 5 mg insulin, 15 mg Epikuron 200, 35 mg sodium ursodeoxycholate and 1 mg sodium citrate, and all these components were dissolved in 1 mL oil phase. Formulations 2 and 3 contained the same components, except that phospholipids were Epikuron 200SH and Epikuron HL50, respectively.

Residual water content

The amount of residual water in the lyophilized product was determined by thermogravimetric analysis (TGA), which was conducted on 3∼5 mg samples in a nitrogen atmosphere at a heating rate of 10°C min^{-1} from 25°C to 150°C using TGA 50 (Shimadzu Co., Japan). The weight loss was used to calculate the amount of residual water.

HPLC analysis of insulin

Before analysis, the oil-based formulations were mixed with purified water and vortexed for 30 s to form emulsions. The emulsions were centrifuged at 50 000 *g* for 30 min. The insulin concentration in the supernatant was determined by two different HPLC methods.

Method 1

The HPLC system consisted of a Shimadzu UV-VIS detector (SPD-10A*vp*) and a solvent delivery pump (LC-10AT*vp*). A reverse-phase Kromasil C18 (200 × 4.6 mm) column packed with 5 μm octadecylsilane was used for assay at 20°C. The data were collected and analysed by CHROM KING Chromatography data system. The mobile phase was acetonitrile–0.2 M (pH 2.3) sodium sulfate (26:74, v/v). The flow rate was maintained at 1 mL min^{-1} and the detection wavelength was set at 266 nm. Insulin had a retention time of ∼33 min and the limit of quantitation of the method was 5 μg mL^{-1} (sample size, 20 μL). The CV% values at different concentrations (50∼500 μg mL^{-1}) were < 5% and the absolute recovery (%) was > 96.5%.

Method 2

The HPLC system consisted of a Perkin Elmer UV/VIS detector (LC 295) and a binary solvent delivery pump (Binary LC pump 250). A Kromasil C8 (5 μm, 200 × 4.6 mm) column was used for assay at 35°C. The flow rate was maintained at 1 mL min^{-1} and the detection wavelength was set at 214 nm. The mobile phases consisted of (A) 0.01 M $(NH_4)_2SO_4$ (pH 2.2, adjusted with conc. H_2SO_4) and (B) acetonitrile containing 0.07% (v/v) trifluoroacetic acid. The mobile phase was initially at 72.0% A and 28.0% B, followed by a gradient after 1.0 min (28.0% B to 30.0% B at 0.18%/min), and a second gradient after 12.0 min (30.0% B to 35.0% B at 0.70%/min). Insulin had a retention time of ∼14 min and the limit of quantitation of the method was 1 μg mL^{-1} (sample size, 20 μL). The CV% values at different concentrations (20∼500 μg mL^{-1}) were < 7% and the absolute recovery (%) was > 95.0%.

In-vivo studies

The animal models employed for testing oral formulations of insulin were Wistar rat (male, about 0.25 kg) and New Zealand albino rabbit (male, about 2 kg), which were provided by the pharmacological laboratory of our school. The study protocol was approved by the Institutional Animal Care and Use Committee, Shenyang Pharmaceutical University, China.

Oral insulin absorption in diabetic rats

Diabetes was induced by intravenous injection of 36 mg kg^{-1} of tetraoxypyrimidine in male Wistar rats. The rats were considered to be diabetic if the basal glucose levels

were more than 10 mmol L^{-1}. Three days after the induction of diabetes and 24 h before experiment, a catheter was implanted in the carotid artery and a fine plastic cannula was surgically inserted into the duodenum of the rat. With the intention of determining the efficacy of oil-based formulations, three formulations were intraduodenally administered to three groups of fasted rats at a dose of 8 mg kg^{-1}. In addition, physiological saline solution was administered to the control group.

Oral insulin absorption in non-diabetic rabbits

At least 10 days before the dosing, a fine plastic cannula was surgically inserted into the duodenum of the rabbit and then brought under the skin onto the back of the rabbit so that the test materials could be injected into the duodenum without distressing the rabbit. An indwelling catheter, which was also brought out through the neck skin, was inserted into a carotid artery for blood sampling.

Before experiments, the rabbits were fasted for 18 h. The formulations were given by instillation via the indwelling cannula into the duodenum.

To test the efficacy of formulation 3 in rabbits, the following preparations were given: control oil solution, which contained the same components as formulation 3 except insulin; control insulin solution at a dose of 1 mg insulin/kg (prepared by dissolving insulin in 50 mM NaOH, and then adjusting the pH value to 8.0); formulation 3 at a dose of 1 mg insulin/kg; formulation 3 at a dose of 2 mg insulin/kg.

To determine in what form insulin was absorbed, insulin emulsion was given at a dose of 1 mg insulin/kg and the blood glucose change was compared with that induced by formulation 3. Insulin emulsion was prepared by mixing control oil solution with control insulin solution.

To determine the best way to deliver ethylenediaminetetraacetic acid (EDTA), two different dosing regimens were used. In the first, formulation 3 was administered at a dose of 2 mg insulin/kg after 40 min pre-delivery of 5 mL of 50 mM EDTA. In the second, 5 mL of 50 mM EDTA and formulation 3 were administered at the same time (insulin was given at a dose of 1 mg kg^{-1}).

Blood sampling and glucose analysis

Blood samples were collected from the carotid artery before the duodenal administration and at different times after dosing. The plasma glucose level at zero time was taken as 100% glucose level. The plasma glucose level was determined by the glucose-oxidase method (Glucose GOD-PAD kit; Beijing Beihua Kangtai Clinical Reagent Company, Beijing, China).

Statistical analysis

The results in all figures are shown as the mean values of plasma glucose levels ± standard deviations (s.d.) of animals of individual groups (n = 8 for diabetic rats; n = 6 for non-diabetic rabbits). In Figure 3, the hypoglycaemic effect of different formulations (formulation 1, 2 and 3) and control in rats at each time point were examined using one-way analysis of variance. In Figure 4, the hypoglycaemic effect of different doses (1 and 2 mg kg^{-1}) and control in non-diabetic rabbits at each time point were examined using one-way analysis of variance too. In both cases, post-hoc comparison of the means of individual groups was performed using Tukey's Honestly Significant Difference test. In Figure 5, 6 and 7, the hypoglycaemic effect of different formulations at each time point was compared using a series of two independent sample *t*-tests. In all cases, $P < 0.05$ was considered to be statistically significant.

Results and Discussion

The possible solubilization mechanism

It is well known that hydrophilic species can not be directly dissolved in oil to form a solution. However, with the aid of certain amphiphiles, it is possible to mask the charges and increase the overall hydrophobicity of hydrophilic drugs, resulting in altered solubility characteristics. Here it is desirable to explore the possible solubilization mechanism implicated in the preparation of oil-based insulin formulations.

As described in Materials and Methods, phospholipids and sodium ursodeoxycholate were dispersed in purified water in an approximately 1:5 molar ratio, resulting in the formation of a mixed micelle solution. When insulin was dissolved in this solution, amphiphiles (phospholipids and bile salts) adopted the correct orientation with respect to insulin, namely with their hydrophilic head groups towards insulin, and with the hydrophobic tails directed away from insulin. When the mixture of insulin and mixed micelles was subjected to freeze-drying, the orientation between amphiphiles and insulin was maintained but the interactions between them may be further enhanced after solvent removal. The loose structure in the lyophilized product permitted the rapid permeation of oil. Therefore, on addition of oil phase, driven by the need to mask the hydrophilic insulin and the head groups of amphiphiles from the oil, the amphiphiles were forced to form a protective sheath between oil and insulin, leading to the formation of reverse micelle-like structures. A schematic representation of this solubilization mechanism can be found in Figure 1.

This structure is, however, different from reverse micelles and water-in-oil microemulsion due to the lack of internal water pool. Our data show that the residual water levels were less than 2% in the lyophilized products. Accordingly, the calculated W_0 (the molar ratio of water to amphiphile) is about 0.8. Reverse micelles usually have a W_0 value of 2.0 or higher (Luisi et al 1988), which means that in oil solution the head groups of amphiphiles are interacting directly with hydrophilic drugs. Thus this specific structure is named anhydrous reverse micelle.

Long-term stability of oil-based insulin formulations

Because insulin is liable to hydrolysis, no liquid preparations are commercially available. However, when it was solubilized in oil, the stability of insulin was greatly enhanced. All oil-based formulations of insulin were

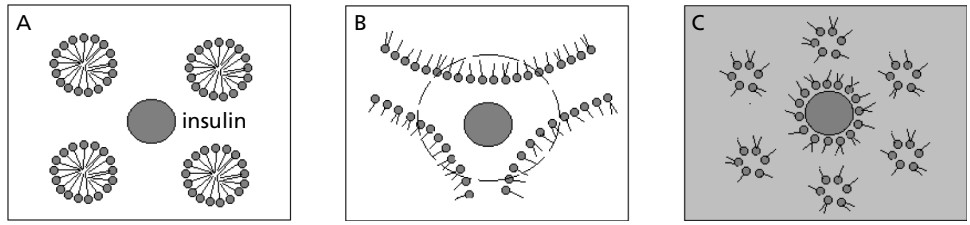

Figure 1 Schematic representation of the solubilization process for the oil-based formulations for oral insulin delivery, showing the solution of insulin and mixed micelles (A), co-dispersion of insulin and amphiphiles after solvent removal (B) and the formation of oil solution on addition of oil (C).

physically and chemically stable at room temperature for at least 12 months. No significant changes in appearance were observed during the course of the stability study. To determine the chemical stability of insulin, two different HPLC methods were used. It was found that there were no detectable degradation products of insulin after storage at room temperature for one year. Therefore, it was concluded that when insulin is solubilized in oil, the stability problems associated with aqueous phase can be resolved.

The release of insulin from oil-based formulations

Because the oil-based formulations were made of surfactants, including bile salt, phospholipids, medium chain partial glycerides and polysorbate 80, they were self-emulsifying drug delivery systems (Pouton 2000). It should be noted, though, that this kind of oil-based formulation can be used as a carrier for hydrophilic drugs other than hydrophobic drugs.

When the oil-based formulation was in contact with water, gentle shaking could lead to the formation of an oil-in-water emulsion. A micrograph of the resulting emulsion is presented in Figure 2.

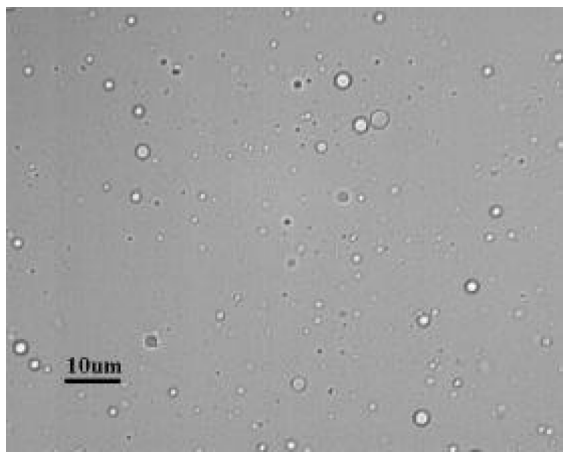

Figure 2 A microscopic view of emulsion obtained by mixing oil-based formulation with water.

To determine the release of insulin from oil phase, formulation 3 was diluted with water in a volume ratio ranging from 1:2 to 1:16. After centrifugation, the resulting emulsions were fractionated into three component phases – oil, supernatant and clear micellar phase. The insulin level in the supernatant liquid was assayed by HPLC (Method 1). It was found that about 60% of insulin was released into the aqueous phase independent of dilution folds. The result shows that on exposure to water, the anhydrous reverse-micelles were destroyed, resulting in insulin release.

If the anhydrous reverse micelles are destroyed, phospholipids and bile salts can also enter aqueous phase to form mixed micelles. Therefore, we can not exclude the possibility that a fraction of the insulin released from oil solution is further solubilized in bile salt/phospholipids mixed micelles because of its high content of non-polar amino acids (28 aa/51 aa).

Oral absorption of insulin in diabetic rats

In this study, three different formulations were used. To screen the optimal formulation for further study, the efficacy of these formulations was tested in-vivo using Wistar rat as animal model. In the control experiment devoid of insulin, there was no modification in blood glucose level (Figure 3). All three formulations resulted in a significant reduction in blood glucose level ($P < 0.05$) for at least 2 h. The order of the hypoglycaemic enhancement effect (Figure 3) was: formulation 3 > formulation 1 > formulation 2. Formulation 3 contained Epikuron HL50, a phospholipid blend that is enriched with lyso-phospholipids. Thus formulation 3 was composed of degradation products of dietary lipids including lyso-phospholipids, monoglycerides (or diglycerides) and fatty acids. Because all these components are physiological permeation enhancers, they can lead to the enhanced absorption of insulin. Moreover, some mechanisms associated with lipid absorption may play a role in oral insulin delivery (Nordskog et al 2001).

Oral absorption of insulin in non-diabetic rabbits

Formulation 3 was further evaluated using New Zealand albino rabbit as animal model. Figures 4–7 exhibit the behaviour of different insulin formulations instilled intraduodenally to rabbits. The efficacy of the formulations

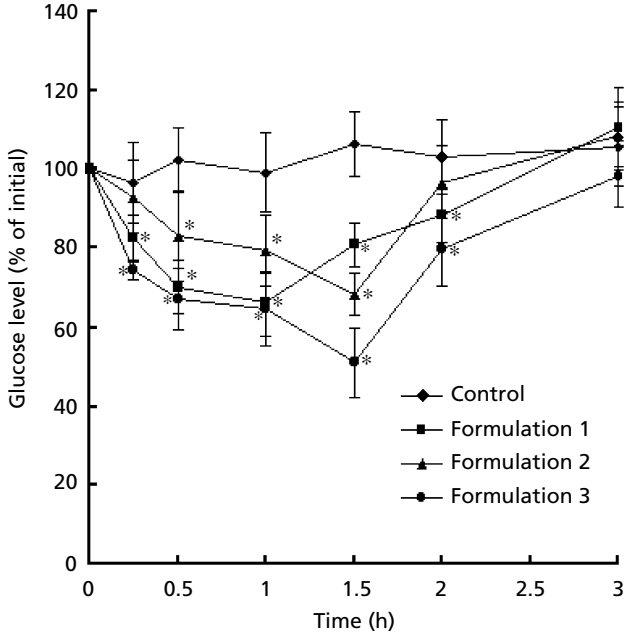

Figure 3 Plasma glucose levels obtained in rats following duodenal administration of control insulin solution or formulations 1, 2 and 3 (mean ± s.d., n = 8); $*P < 0.05$ vs control.

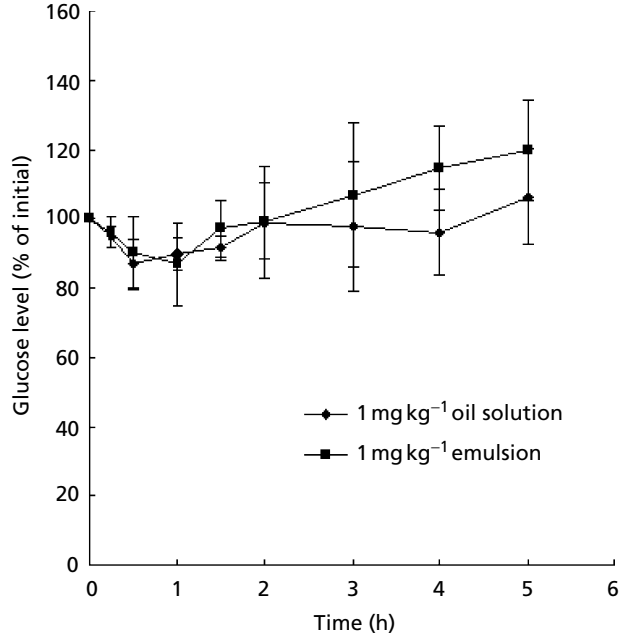

Figure 5 Plasma glucose levels achieved in rabbits following duodenal administration of insulin oil solution and insulin emulsion (mean ± s.d., n = 6); all P values > 0.05, insulin oil solution vs insulin emulsion.

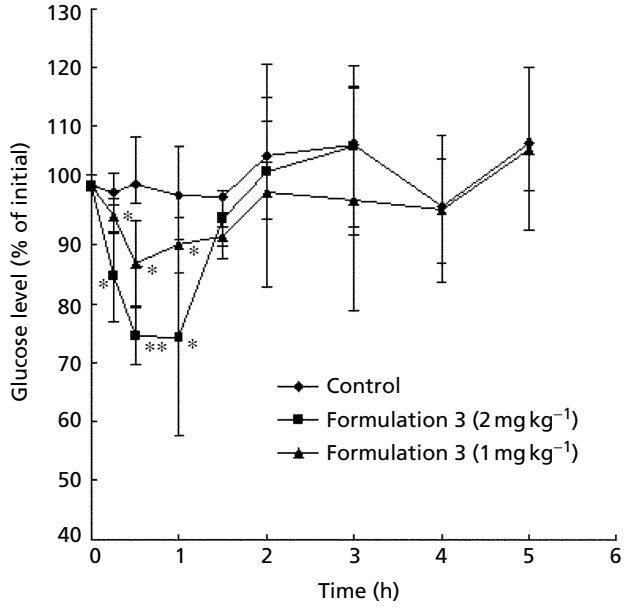

Figure 4 Plasma glucose levels obtained in rabbits following duodenal administration of control insulin oil solution or formulation 3 (1 or 2 mg kg^{-1}) (mean ± s.d., n = 6); $*P < 0.05$, $**P < 0.001$ vs control oil solution.

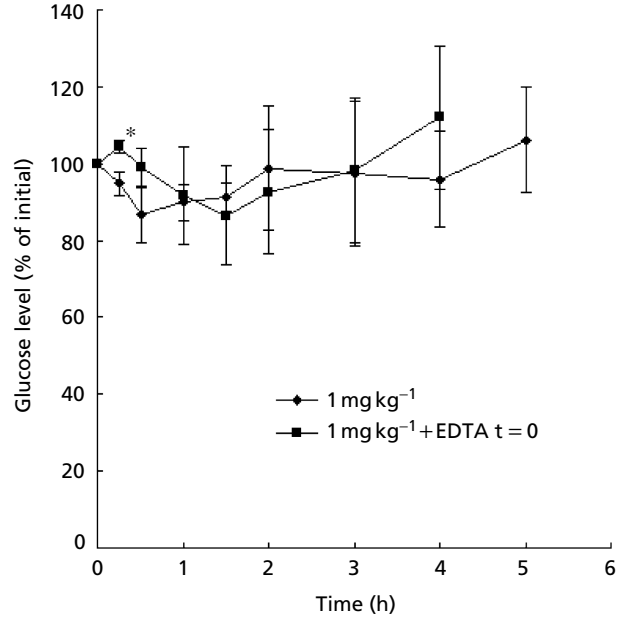

Figure 6 Plasma glucose levels achieved in rabbits following duodenal administration of formulation 3, or co-delivery of formulation 3 and EDTA at the same time (mean ± s.d., n = 6); $*P < 0.05$ (0.25 h), with EDTA vs without EDTA.

was assessed by measuring the plasma glucose concentration, too.

The administration of control oil solution without insulin to rabbits did not cause any modification in blood glucose levels during the experimental period (Figure 4), indicating that the rabbits were not stressed by the administration procedure and blood sampling. The administration of insulin control solution at a dose of 1 mg kg^{-1} had

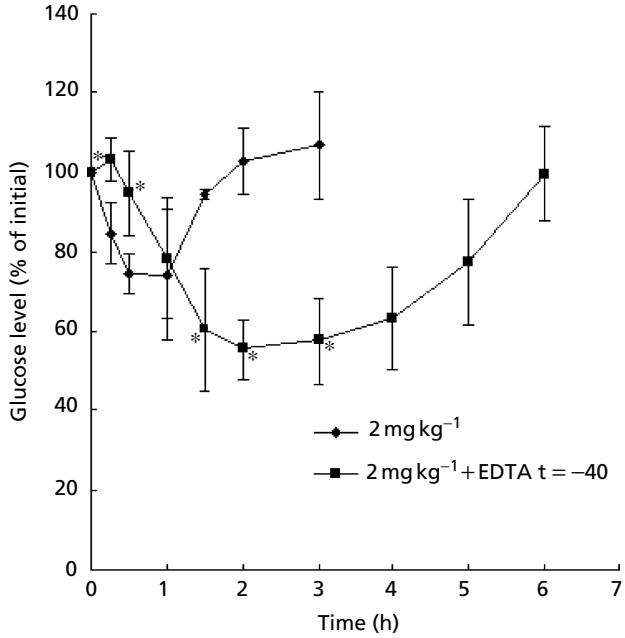

Figure 7 Plasma glucose levels achieved in rabbits following duodenal administration of formulation 3 with and without pre-delivery of EDTA. Na_2EDTA was delivered 40 min before the delivery of formulation 3 (mean ± s.d., n = 6); $^*P < 0.05$, with EDTA vs without EDTA.

no obvious influence on the glucose level, too (data not given in order to improve the clarity of Figure 4). The same figure shows that the administration of formulation 3 (1 mg kg^{-1}) resulted in a decrease in blood glucose level of 15% at 30 min post-administration. Moreover, the administration of formulation 3 at a dose of 2 mg kg^{-1} led to an increased systemic absorption of insulin. Indeed, the blood glucose level fell to 75% of basal level (maximum glucose reduction). The plasma glucose level was significantly different ($P < 0.05$) to that of the control group for at least 1 h. These results clearly prove the ability of insulin oil solution to enhance the absorption of insulin and the efficacy of formulation 3 showed dose dependence when administered to rabbits at increasing dosage. Furthermore, these data indicate that insulin was released from oil solution in its active form, thus revealing that the preparation method was not harmful to this peptide.

To investigate whether or not the solubilization of insulin in oil in the form of anhydrous reverse micelles has a role in improving the absorption of insulin, the effect of insulin oil solution was compared with that of insulin emulsion. Rabbits were treated with an emulsion containing the same dose of insulin as formulation 3 (1 mg kg^{-1}). Results clearly showed that the decrease in plasma glucose induced by the administration of formulation 3 was similar to that obtained following the instillation of insulin emulsion (Figure 5).

Because insulin emulsion was prepared by mixing the corresponding excipients, it did not involve the formation of anhydrous reverse micelles. The similar efficacy of insulin oil solution and insulin emulsion revealed that insulin was not absorbed in the form of anhydrous reverse micelles. This was in agreement with the fact that on exposure to water the anhydrous reverse micelles were destroyed and a large fraction of insulin released into aqueous phase. Hence it is concluded that the oral absorption of insulin is independent of anhydrous reverse micelles, but to achieve effective solubilization, this structure is indispensable. Although insulin oil solution has the same efficacy as insulin emulsion, it still has advantages over the emulsion because it can resolve the stability problems of insulin associated with aqueous phase.

Although in-vivo studies have proved the efficacy of formulation 3, it is still desirable to further improve the oral insulin delivery using other strategies. As mentioned above, when formulation 3 was exposed to aqueous phase, a large fraction of insulin was released from the oil phase, which might be hydrolysed by digestive enzymes because formulation 3 did not contain any enzyme inhibitor. Therefore, to overcome enzyme barriers, it is necessary to use enzyme inhibitors in conjunction with formulation 3.

In our study, EDTA was used because it is a strong chelating agent that can remove ions required by digestive enzymes during proteolysis, thereby substantially reducing the proteolytic activity of the enzymes (Bernkop-Schnurch & Krajicek 1998).

Unfortunately, it was found that co-delivery of 5 mL Na_2EDTA (50 mM) and formulation 3 (1 mg insulin/kg) at the same time did not result in significant enhancement of the hypoglycaemic effect of formulation 3 (Figure 6). In addition, the co-delivery of EDTA induced a delayed absorption phase of insulin, with maximum glucose reduction at 90 min post-administration instead of at 30 min.

However, pre-delivery of 5 mL Na_2EDTA (50 mM) before duodenal administration of formulation 3 resulted in an unexpectedly high intestinal absorption of insulin. When Na_2EDTA was delivered 40 min before the delivery of formulation 3, a marked difference in insulin absorption occurred (Figure 7). When only formulation 3 was administered at a dose of 2 mg insulin/kg, the AUC (% glucose reduced, the areas above basal levels were not included) was 28.5 ± 14.7. In contrast, pre-delivery of Na_2EDTA before administration of formulation 3 (2 mg insulin/kg, also) resulted in an AUC value of 167.1 ± 72.3. Similarly, pre-delivery of EDTA also induced delayed insulin absorption (Figure 7).

Why did pre-delivery of EDTA lead to the enhanced absorption of insulin? An explanation for this phenomenon is that there is a lag time before EDTA can take effect. Perhaps it takes some time for EDTA to remove ions, resulting in the transient inactivation of digestive enzymes. In addition, EDTA has been shown to enhance the paracellular transport by depletion of Ca^{2+} in the extracellular space, including the tight junctional region (Tomita et al 1994, 1996), which also needs time to realize.

Accordingly, there is no enhancement of insulin absorption if EDTA is delivered at the same time as formulation 3, and an improvement occurs where there is a delay between the administration of EDTA and formulation 3.

However, it should be noted that EDTA might also serve other functions. For example, EDTA might affect peristalsis, leading to reduced intestine mobility. If this is the case, it is not surprising that administration of EDTA induced a delayed absorption phase of insulin as shown in Figures 6 and 7. It is known that insulin can be absorbed from the ileum, the ascending colon and the descending colon (Morishita et al 1993). Accordingly, if peristalsis is inhibited, the transit time from duodenum to ileum will increase, leading to the delayed absorption of insulin. The mechanism behind peristalsis inhibition may be related to neurohumoral control of gastrointestinal mobility (Olsson & Holmgren 2001; Hansen 2003) and needs further investigation.

Conclusions

Insulin can be solubilized in oil in the form of anhydrous reverse micelles and the resulting oil solutions are physically and chemically stable. The oral absorption of insulin is independent of the existence of anhydrous reverse micelles but to achieve effective solubilization, this structure is indispensable. Furthermore, by pre-delivery of EDTA before the administration of insulin oil solution, the therapeutic effect of insulin can be greatly enhanced.

References

Bernkop-Schnurch, A. (1998) The use of inhibitory agents to overcome the enzymatic barrier to perorally administered therapeutic peptides and proteins. *J. Control. Release* **52**: 1–16

Bernkop-Schnurch, A., Krajicek, M. E. (1998) Mucoadhesive polymers as platforms for peroral peptide delivery and absorption: synthesis and evaluation of different chitosan-EDTA conjugates. *J. Control. Release* **50**: 215–223

Cournarie, F., Auchere, D., Chevenne, D., Lacour, B., Seiller, M., Vauthier, C. (2002) Absorption and efficiency of insulin after oral administration of insulin-loaded nanocapsules in diabetic rats. *Int. J. Pharm.* **242**: 325–328

Damge, C., Vranckx, H., Balschmidt, P., Couvreur, P. (1997) Poly(alkyl cyanoacrylate) nanospheres for oral administration of insulin. *J. Pharm. Sci.* **86**: 1403–1409

Fix, J. A. (1996) Strategies for delivery of peptides utilizing absorption-enhancing agents. *J. Pharm. Sci.* **85**: 1282–1285

Hansen, M. B. (2003) Neurohumoral control of gastrointestinal motility. *Physiol. Res.* **52**: 1–30

Hosny, E. A., Ghilzai, N. M. K., Elmazar, M. M. (1997) Promotion of oral insulin absorption in diabetic rabbits using pH-dependent coated capsules containing sodium cholate. *Pharm. Acta Helv.* **72**: 203–207

Hosny, E. A., Al-Shora, H. I., Elmazar, M. M. A. (2002) Oral delivery of insulin from enteric-coated capsules containing sodium salicylate: effect on relative hypoglycemia of diabetic beagle dogs. *Int. J. Pharm.* **237**: 71–76

Kisel, M. A., Kulik, L. N., Tsybovsky, I. S., Vlasov, A. P., Vorob'yov, M. S., Kholodova, E. A., Zabarovskaya, Z. V. (2001) Liposomes with phosphatidylethanol as a carrier for oral delivery of insulin: studies in the rat. *Int. J. Pharm.* **216**: 105–114

Lowman, A. M., Morishita, M., Kajita, M., Nagai, T., Peppas, N. A. (1999) Oral delivery of insulin using pH-responsive complexation gel. *J. Pharm. Sci.* **88**: 933–937

Luisi, P. L., Giomini, M., Pileni, M. P., Robinson, B. H. (1988) Reverse micelles as hosts for proteins and small molecules. *Biochim. Biophys. Acta* **947**: 209–246

Morishita, M., Morishita, I., Takayama, K., Machida, Y., Nagai, T. (1993) Site-dependent effect of aprotinin, sodium caprate, Na_2EDTA and sodium glycocholate on intestinal absorption of insulin. *Biol. Pharm. Bull.* **16**: 68–72

Muramatsu, K., Maitani, Y., Nagai, T. (1996) DPPC liposomes with soybean-derived sterols and cholesterol as a carrier for the oral administration of insulin in rats. *Biol. Pharm. Bull.* **19**: 1055–1058

New, R. R. C., Kirby, C. J. (1997) Solubilization of hydropohilic drugs in oily formulations. *Adv. Drug Deliv. Rev.* **25**: 59–69

Nordskog, B. K., Phan, C. T., Nutting, D. F., Tso, P. (2001) An examination of the factors affecting intestinal lymphatic transport of dietary lipids. *Adv. Drug Deliv. Rev.* **50**: 21–44

Olsson, C., Holmgren, S. (2001) The control of gut motility. *Comp. Biochem. Phys. A.* **128**: 481–503

Pillai, O., Panchagnula, R. (2001) Insulin therapies – past, present and future. *Drug Discov. Today* **6**: 1056–1061

Pouton, C. W. (2000) Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and 'self-microemulsifying' drug delivery systems. *Eur. J. Pharm. Sci.* **11** (Suppl. 2): S93–S98

Radwan, M. A. (2001) Enhancement of absorption of insulin-loaded polyisobutylcyanoacrylate nanospheres by sodium cholate after oral and subcutaneous administration in diabetic rats. *Drug Dev. Ind. Pharm.* **27**: 981–989

Shimoda, J., Onishi, H., Machida, Y. (2001) Bioadhesive characteristics of chitosan microspheres to the mucosa of rat small intestine. *Drug Dev. Ind. Pharm.* **27**: 567–576

Tomita, M., Hayashi, M., Awazu, S. (1994) Comparison of absorption-enhancing effect between sodium caprate and disodium ethylenediaminetetraacetate in Caco-2 cells. *Biol. Pharm. Bull.* **17**: 753–755

Tomita, M., Hayashi, M., Awazu, S. (1996) Absorption-enhancing mechanism of EDTA, caprate, and decanoylcarnitine in Caco-2 cells. *J. Pharm. Sci.* **85**: 608–611

Tozaki, H., Nishioka, J., Komoike, J., Okada, N., Fujita, T., Muranishi, S., Kim, S., Terashima, H., Yamamoto, A. (2001) Enhanced absorption of insulin and ($Asu^{1,7}$) eel-calcitonin using novel azopolymer-coated pellets for colon-specific drug delivery. *J. Pharm. Sci.* **90**: 89–97

Trehan, A., Ali, A. (1998) Recent approaches in insulin delivery. *Drug Dev. Ind. Pharm.* **90**: 589–597

Watnasirichaikul, S., Rades, T., Tuker, I. G., Davies, N. M. (2002) In-vitro release and oral bioactivity of insulin in diabetic rats using nanocapsules dispersed in biocompatible microemulsion. *J. Pharm. Pharmacol.* **54**: 473–480