Enhancement of the intranasal delivery of insulin via a novel mucoadhesive Carbopol gel

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Abstract

Objectives The objectives of this study were to develop an intranasal insulin gel using Carbopol homogenization rather than neutralization and to examine the effectiveness of the gel compared with a subcutaneous injection.

Methods Four factors, namely the molecular weight of polyethylene glycol (PEG), the concentration of Carbopol, the temperature of preparation and the type of absorption enhancer, were evaluated for their effect on viscosity and in-vitro insulin release. Bioavailability of insulin from selected formulations was compared with an intranasal solution and subcutaneous injection in rabbits.

Key findings Increasing the molecular weight of PEG and Carbopol concentration increased the gel viscosity and changed the release mechanism from diffusion to case II transport. Applying heat during preparation resulted in a lower viscosity gel and increased the in-vitro insulin release. Among the two enhancers studied, sodium deoxycholate resulted in a higher viscosity gel than Tween 80. *In vivo*, the intranasal gel showed a stronger and longer hypoglycaemic effect with 1.7- and 3.1-fold higher maximum decrease in blood glucose level and area above the curve, respectively, compared with the subcutaneous injection.

Conclusions The homogenized Carbopol intranasal gel could be an efficient noninvasive way for insulin delivery but selection of gel components and method of preparation are critical for achieving the most desired effect.

Keywords Carbopol gel; insulin; intranasal delivery; permeation enhancer; polyethylene glycol

Introduction

Several methods have been proposed as convenient alternatives to subcutaneous injection for the delivery of insulin in the treatment of diabetes.[1] One method that has been widely investigated is intranasal delivery. This is a non-invasive route that would potentially improve patient compliance compared with subcutaneous injection. It is also less liable to cause episodes of hypoglycaemia and does not produce peripheral hyperinsulinaemia, which has been involved in the exacerbation of the macrovascular complications of diabetes.

In addition to its effect in managing the blood glucose level, intranasal insulin might act as a vaccine to induce protective immune cells to counteract the primary mediators of β-cell destruction (CD4+ and CD8+ T cells),[2] thereby hindering the progress of diabetes.[3] Intranasal insulin also has rapid direct access to the central nervous system (within 15 min) to offer other therapeutic benefits such as improving memory in patients with early Alzheimer's disease. This improvement in memory possibly occurs by augmenting the low brain levels of insulin encountered in patients and modulating the plasma β-amyloid and cortisol levels.[4] However, insulin exhibits weak absorption from the nasal cavity along with a pharmacokinetic profile that bears close resemblance to that of the intravenous injection in terms of its rapid onset and short-acting effect. Therefore, effective insulin absorption via the nasal route is unlikely to occur without the presence of absorption enhancers, which modulate nasal epithelial permeability to insulin and/or prolong the residence time of the drug formulation in the nasal cavity.[5]

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Several studies have reported the enhancement of intranasal delivery of insulin using inserts,[6] microspheres,[7] nanoparticles [8] and gels.[9] D'Souza *et al.*[10] developed an intranasal gel for insulin delivery by neutralizing an aqueous dispersion of Carbopol with alkali. However, this method suffered from precipitation of insulin in the gel upon neutralization.

The objective of this study was to develop an optimal Carbopol gel for intranasal delivery of insulin via a method that would overcome the issue of insulin precipitation in the gel upon neutralization of Carbopol. Moreover, it aimed to enhance the release and intranasal absorption of insulin in rabbits together with providing a prolonged controlled hypoglycaemic effect.

Materials and Methods

Materials

Highly purified recombinant human insulin crystal was obtained from Tonghua Dongbao Pharmaceutical Co., Ltd (China). Carbopol 934 and polyethylene glycol (PEG 400 and PEG 600) were received from BF Goodrich (Cleveland, OH, USA) and Alpha Chemika (Mumbai, India), respectively. Glycerin was obtained from El Gomhoria Company, Cairo, Egypt.

Experimental design

Investigations were first performed for selection of the factors to include in the study as well as their levels. Selection of the gelation media (factor X_1) was initially performed by visually inspecting gel prepared in water, glycerin, PEG 400 or PEG 600 using a visual inspection machine (Bosch, Germany). The levels of the gelling agent Carbopol 934 (factor X_2) were selected based on the consistency of gels prepared with concentrations of Carbopol over the range of 0.5 to 6% w/v. The preparation condition under which the gels were formed (hot or cold) was selected as a third factor (factor X_3).

Two types of permeation enhancers (factor X_4) were used, namely sodium deoxycholate and Tween 80. Sodium deoxycholate (1% w/v) was chosen due to the fact that it is an endogenous bile salt reported to have a 5- to 6-fold higher permeation enhancing property than sodium glycocholate, together with much lower toxicity.[11] Tween 80 was selected from an in-vivo study conducted to compare the permeation enhancing properties of Tween 80 (1% w/w), β-cyclodextrin (1% w/w) and Myrj 53 (0.5% w/w) for the intranasal delivery of insulin solution in 18 healthy male rabbits.

Determination of the appropriate level for each of the four factors (X_1–X_4) was done by performing a full $3^1.2^3$ factorial experiment where 24 gel formulations were prepared.

Preparation of Carbopol gel

The gels were prepared using the method adapted by Bonacucina *et al.*,[12] where accurately weighed amounts of Carbopol corresponding to concentrations of 3, 4 and 5% were sprinkled on the surface of the gelation medium and dispersed by mixing with a magnetic stirrer. The dispersions were then homogenized for 5 min at 27 000 rev/min (Homogenizer, type 302; Mechanika Precyzyjna, Poland), degassed by sonication and kept at rest for 1 day before use.

Another set of gels were prepared in the same way but heated after complete homogenization to 70°C with continuous stirring for 30 min. The gels prepared were then allowed to stand for 1 day before use.

Sodium metabisulfite (0.01% w/v) as an antioxidant and benzoic acid (0.05% w/w) as a preservative were added to the stirred liquid phase before the incorporation of Carbopol. The permeation enhancer was then added to the gels formed followed by 1 ml Sorenson's buffer (pH 7.4) containing an amount of insulin equivalent to 100 IU.

In-vitro evaluation of gels
Rheological measurement
The viscosity of 1 g of each of the freshly prepared gels was determined using a cone and plate Brookfield viscometer (model HBDV-I; Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) at 25°C and angular velocities ranging from 0.3 to 4 rev/min with a 30-s wait for each rev/min to determine the flow type. The data obtained was analysed using the power law:

$$\sigma = K\gamma^P \tag{1}$$

where, σ is the shear stress, K is the consistency index, γ is the shear rate and P is the power law index.

According to the power law model, P values of less than, equal to or greater than unity would be indicative of pseudoplastic, Newtonian or dilatant flow behaviour, respectively.

In-vitro release study
Insulin release from the gel formulations was examined in triplicates using USP-XXIV apparatus 1. An accurately weighed amount of each preparation was introduced into a basket that was then immersed in a vessel containing 200 ml of Sorenson's phosphate buffer pH 7.4 at 37°C and stirred at 50 rev/min. Samples (3 ml) were withdrawn at predetermined time intervals up to 8 h and replaced with an equal volume of fresh medium. The samples were filtered through a 0.45-μm membrane filter and assayed spectrophotometrically for insulin at a wavelength of 277 nm.

The extent of release was determined by measuring the release efficiency after 8 h ($RE_{8\,h}$) according to the following equation:[13]

$$RE = \int_0^t \frac{y\,dt}{y_{100}t} \times 100 \tag{2}$$

where the integral in Equation 2 is the area under the dissolution curve up to the dissolution time t and y_{100} is the area of the rectangle described by 100% dissolution at the same time.

Kinetics of insulin release from the gels was evaluated by finding the best fit of the release data to the zero order and Higuchi models (Equations 3 and 4, respectively).

$$Q_t = k_0 t \tag{3}$$

where Q is the cumulative amount released at time t and k_0 is the zero order rate constant.

$$Q_t = k_H t^{1/2} \tag{4}$$

where k_H is the Higuchi's model rate constant.

The mechanism of release was evaluated by plotting log cumulative percent release versus log time for the first 60% of drug release according to the Korsmeyer-Peppas model (Equation 5) and calculating the exponent n from the slope of the straight line obtained.

$$Q_t/Q_\infty = kt^n \qquad (5)$$

where Q_t/Q_∞ is the fraction of drug released at time t, k is the kinetic release constant and n is an exponent characterizing the release mechanism.

Values of $n \leq 0.5$ were characteristic of a Fickian diffusion release mechanism, whereas values falling between 0.5 and 1 were indicative of a non-Fickian model (anomalous transport). However, a value of unity or higher would be expected for zero order release (case II transport) or super case II transport, respectively.

Effect of storage

The selected insulin-loaded gel formulations were stored under cold conditions (3–5°C) for 6 months. Gel samples were withdrawn and evaluated for clarity, insulin content, viscosity and release profile at time intervals of 1, 2, 4 and 6 months. The difference between the release profiles of stored and fresh samples was calculated as follows:[14]

$$S_d = \frac{\sum_{t=1}^{n-1} \left| \log\left((AUC_{Ft})/(AUC_{St}) \right) \right|}{n-1} \qquad (6)$$

where n is the number of data points collected during the in-vitro release test, while AUC_{Ft} and AUC_{St} are the areas under the release curves of fresh gel and samples stored to time t, respectively. The percentage difference between the two release profiles is expected to increase with the increase in S_d value.

In-vivo study

The in-vivo study was performed using rabbits according to the institutional rules of Cairo University. The study protocol was approved by the research ethics committee and was in compliance with the Guidelines of the European Community and the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Bioavailability of two selected intranasal insulin-loaded gels was compared with that of an intranasal solution and a subcutaneous insulin injection. Moreover, the effect of absorption enhancers on the bioavailability of insulin from the gels was evaluated.

Twenty four healthy male rabbits (2.5–3 kg) were fasted overnight with free access to water before being randomly assigned to four groups to receive one of the following treatments: intranasal insulin solution (200 μl containing 100 IU/ml insulin and 1% Tween in Sorenson's buffer pH 7.4); subcutaneous insulin solution (7.5 IU/kg) as a control; an insulin-loaded hydrogel containing Tween or sodium deoxycholate as an absorption enhancer.

Blood samples (1 drop each) were drawn from the ear vein at time intervals of 0.5, 1, 2, 3, 4, 5, 6, 7 and 24 h. Blood glucose levels were measured using an Accu-Chek Easy blood glucose monitor and test strips (Boehringer Mannheim Corp., Indianapolis, IN, USA). Blood glucose levels at 1, 0.5, and 0 h

before administration were measured and the mean of the three values was considered as the baseline glucose level for normalization of the rest of the values.

The normalized blood glucose values were plotted versus time to obtain a blood glucose level–time profile. In this study the area above the blood glucose level–time curve for 24 h (AAC_{0-24}) was calculated using the linear trapezoidal rule[15] and used as a reflection of the $AUC_{(0-24)}$ for the insulin level–time curve.[16] In addition, other pharmacokinetic parameters such as the maximum percent decrease in blood glucose levels (C_{max}) and the time required to reach that level (t_{max}) were determined and used to evaluate the efficacy of the formulations.

Statistical analysis

The effects of the factors studied on viscosity (measured at a fixed shear rate of 0.3/s) and in-vitro release parameters (n value, and %RE) were evaluated statistically by analysis of variance at $P < 0.01$ using JMP software (version 4.0.4; SAS Institute, Cary, NC, USA). The estimated in-vivo $AAC_{(0-24)}$, C_{max} and t_{max} values for the different formulations were presented as mean $\pm$ s.d. and compared statistically using one-way analysis of variance at $P < 0.05$.

Results

Selection of gel components
Gelation media
Transparent clear homogenized gels were obtained with the use of PEG 400 or PEG 600 as gelation media, whereas in water or glycerin white turbid gels were formed.

Carbopol concentration
Visual inspection showed that under cold and hot conditions clear homogenous gels could be obtained but the Carbopol concentration was critical. At Carbopol concentrations below 3% w/w gelation did not occur, whereas very viscous, difficult to use gels were obtained above the 6% w/w concentration. Therefore, three levels of Carbopol over the range of 3 to 6% w/w were selected.

Permeation enhancer
An initial in-vivo study was performed to compare the permeation enhancing property of insulin solutions containing Tween 80, β-cyclodextrin and Myrj 53. This study showed that the $AAC_{(0-24)}$ value for the Tween 80 solution (13.09 $\pm$ 1.08 μg h/ml) was significantly the highest (Figure 1). The β-cyclodextrin solution followed with an $AAC_{(0-24)}$ value of 8.76 $\pm$ 0.41 μg h/ml, whereas Myrj 53 had the lowest $AAC_{(0-24)}$ (2.68 $\pm$ 0.26 μg h/ml). Moreover, the maximum percent decrease in blood glucose level value for the Tween solution (38.2 $\pm$ 1.11 μg/ml) was significantly higher than for β-cyclodextrin and Myrj 53 (24.766 $\pm$ 1.098 and 7.66 $\pm$ 1.36, respectively). These results indicated that the best permeation enhancement was obtained with Tween 80.

In-vitro evaluation of the prepared gels
Rheological properties
All the prepared gel formulations showed power law index values (P) ranging between 0.67 and 0.84, which indicated
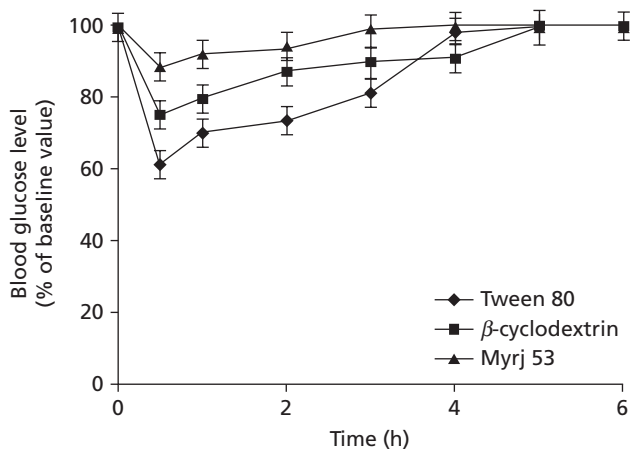
Figure 1 Effect of permeation enhancers. Blood glucose levels (% of baseline value) in rabbits receiving intranasal insulin solutions with different permeation enhancers ($n = 6$).
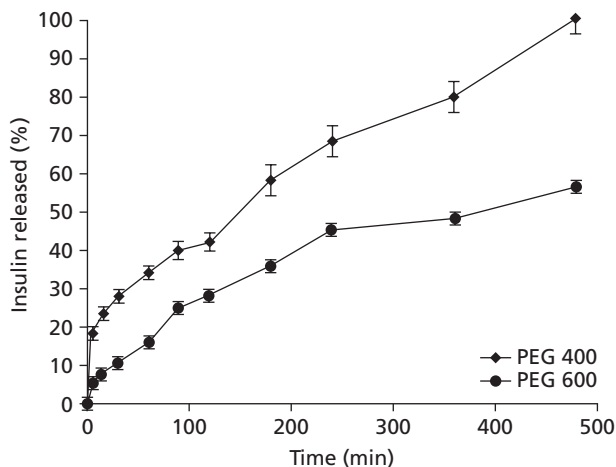

Figure 2 Effect of polyethylene glycol. Effect of PEG molecular weight on the in-vitro release of insulin from gels prepared with 4% gelling agent under hot conditions.
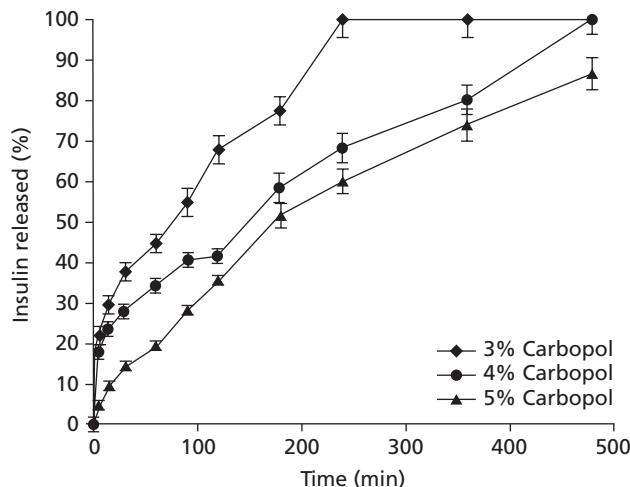

Figure 3 Effect of Carbopol concentration. Effect of Carbopol concentration on the in-vitro release of insulin from gels prepared with PEG 400 under hot conditions.

that all the gels exhibited shear thinning behaviour independent of any of the variables being investigated. Statistical evaluation of the viscosity data collected at a shear rate of 0.3/s showed that the molecular weight of PEG, the Carbopol concentration and the preparation condition had significant effects on viscosity, whereas the permeation enhancer had no effect ($P \leq 0.01$). Moreover, the only interaction having a significant effect on viscosity was the one occurring between the PEG molecular weight and the Carbopol concentration. A synergistic increase in gel viscosity was obtained by increasing the molecular weight of PEG and the Carbopol concentration (data not shown). The decrease in viscosity from 333×10^4 cPs for F21 to 280×10^4 cPs for F23, where the two formulations varied only in the preparation condition, was typical for the effect of heat on the viscosity of the homogenized gels. The higher viscosity observed for F24 (312×10^4 cPs) compared with F23 (280×10^4 cPs) showed the typical effect of the absorption enhancer used on viscosity, where sodium deoxycholate imparted higher viscosity to the gel compared with Tween 80.

In-vitro release study
Release of insulin was sustained from all the gels prepared as indicated by RE_{8h} values ranging from 28.2 to 81.4 (data not shown). This was probably due to the viscosity of the gels and their gradual swelling in the aqueous medium. Only three of the four main variables studied, namely the PEG molecular weight, the concentration of Carbopol and the preparation condition, had significant effects on RE_{8h}. The increase in PEG molecular weight or Carbopol concentration caused a decrease in RE_{8h} and retarded the release of insulin (Figures 2 and 3, respectively).

Heating the gels at the end of the preparation step significantly increased the RE_{8h} values and enhanced the release of insulin (Figure 4).

Release kinetics
Upon fitting the first 60% of the in-vitro insulin release to the Higuchi and zero order models, a better linearity for most of the gels was obtained with the Higuchi model, as indicated by the higher determination coefficient (r^2) presented in Table 1. However, formulations containing PEG 600 with 5% Carbopol had a better fit to the zero order plot.

Mechanism of insulin release
Release plots according to the Korsmeyer-Peppas equation showed good linearity for all the formulations, with r^2 ranging from 0.9141 to 0.9967. The n exponent of the Korsmeyer-Peppas equation was lower than 0.5 for gel formulations having low molecular weight PEG and the lowest concentration of Carbopol (3%) (Table 1). This indicated that drug diffusion through the gel was the rate limiting step for the release of drug from these gels (formulations 1–4). However, n values falling between 0.5 and 0.89 were obtained by increasing the Carbopol
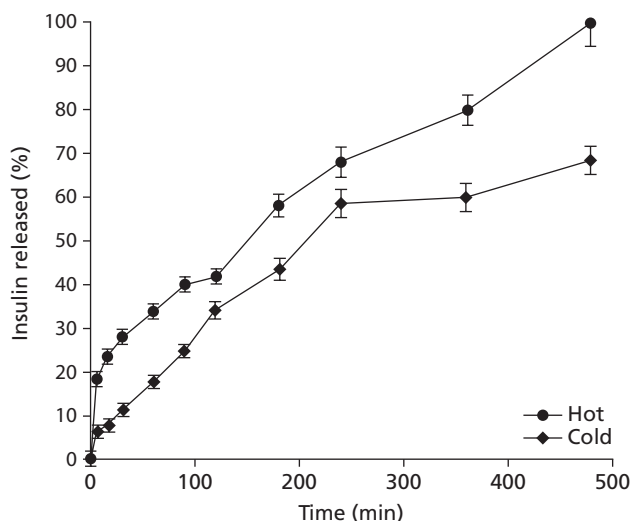
Figure 4 Effect of preparation condition. Effect of preparation condition on the in-vitro release profiles of insulin loaded gels prepared with PEG 400 and 4% Carbopol.

concentration from 3% to 4 and 5% while maintaining the same low molecular weight PEG (formulations 5–12). This reflected an anomalous release mechanism, which was affected by both drug diffusion and swelling of the gel. For gels containing the high molecular weight PEG (PEG 600), the presence of a 3% Carbopol concentration showed an anomalous release mechanism (formulations 13–16).

Increasing the Carbopol concentration to 4 and 5% resulted in n values higher than 0.89, except for 4% Carbopol gel formulations prepared under hot conditions (formulations 19 and 20). The two latter formulations showed values for the n exponent lower than 0.89 (0.81 and 0.87 for the Tween 80 and sodium deoxycholate containing gels, respectively). The above results indicated that with the higher molecular weight PEG, using Carbopol concentrations of 4 and 5% changed the release mechanism to case II transport, that is zero order release kinetics, and super case II transport ($n > 1$). In both of these cases, the release mechanism was mainly dependent on swelling and relaxation of the polymer. However, swelling of the 4% Carbopol gel seemed to be faster if prepared under hot conditions, thus resulting in a release mechanism that is more anomalous (i.e. dependent on both drug diffusion and gel swelling rather than the latter alone).

Statistical analysis showed that the four factors examined exerted significant effects on the n exponent value. However, there was only one significant interaction, which was that between the PEG molecular weight and the Carbopol concentration. At the lower molecular weight PEG, the n value increased from 0.3 to 0.65 by increasing the Carbopol concentration, indicating a change in the release mechanism from Fickian diffusion to anomalous release. However, at the high molecular weight PEG the rise in the n value occurred from about 0.55 up to 1.16 with the increase in the Carbopol concentration. This revealed a change in the release mechanism from anomalous, at the lower Carbopol level (3%), to case II and super case II transport for the higher levels (4 and 5%).

Table 1 Variables evaluated and the release kinetics results of the 3.2^3 full factorial design

Formulation no.	Gelation medium	Carbopol concentration	Preparation condition	Permeation enhancer	Higuchi model r^2	Zero order r^2	Korsmeyer-Peppas n exponent	Release mechanism
1	PEG 400	3%	Cold	Tween	0.991	0.920	0.38	Fickian diffusion
2				Sodium deoxycholate	0.987	0.956	0.38	Fickian diffusion
3			Hot	Tween	0.973	0.985	0.28	Fickian diffusion
4				Sodium deoxycholate	0.973	0.984	0.31	Fickian diffusion
5	PEG 400	4%	Cold	Tween	0.981	0.884	0.60	Anomalous
6				Sodium deoxycholate	0.974	0.904	0.68	Anomalous
7			Hot	Tween	0.968	0.976	0.58	Anomalous
8				Sodium deoxycholate	0.976	0.983	0.61	Anomalous
9	PEG 400	5%	Cold	Tween	0.959	0.875	0.61	Anomalous
10				Sodium deoxycholate	0.942	0.906	0.63	Anomalous
11			Hot	Tween	0.973	0.895	0.62	Anomalous
12				Sodium deoxycholate	0.990	0.959	0.66	Anomalous
13	PEG 600	3%	Cold	Tween	0.941	0.911	0.52	Fickian diffusion
14				Sodium deoxycholate	0.987	0.956	0.55	Anomalous
15			Hot	Tween	0.938	0.855	0.55	Anomalous
16				Sodium deoxycholate	0.953	0.954	0.56	Anomalous
17	PEG 600	4%	Cold	Tween	0.981	0.917	0.91	Anomalous
18				Sodium deoxycholate	0.990	0.962	0.98	Anomalous
19			Hot	Tween	0.984	0.946	0.81	Anomalous
20				Sodium deoxycholate	0.984	0.926	0.87	Anomalous
21	PEG 600	5%	Cold	Tween	0.935	0.933	1.05	Case II transport
22				Sodium deoxycholate	0.935	0.995	1.16	Super case II transport
23			Hot	Tween	0.947	0.976	1.01	Case II transport
24				Sodium deoxycholate	0.979	0.975	1.04	Case II transport

Effect of storage

The selected gel formulations retained their clarity and insulin content (98.3–102.3%) for up to 6 months. Moreover, no marked changes were recorded in their rheological properties and the difference in the release profiles (S_d) for the 6-month period was considerably close to zero (0.008–0.027), indicating a relatively good similarity between the release profiles.

In-vivo evaluation

After subcutaneous administration of insulin in rabbits, there was a biphasic decrease in the serum glucose concentration that remained low for 6 h (Figure 5). The two peaks displayed by the subcutaneous insulin profile were at 0.5 and 3 h. Moreover, the mean maximum percent decrease in blood glucose level and the mean $AAC_{(0-24)}$ values for this route were 24.08% and 12.04 μg h/ml, respectively (Table 2). Administration of the intranasal insulin solution resulted in a rapid monophasic decrease in blood glucose level with a nadir (61.7% of the baseline value) occurring at 0.5 h after dosing and returning to the basal level within 4 h (Figure 5). This rapid absorption shows a pharmacokinetic profile similar to the intravenous insulin and the postprandial endogenous insulin secretion by the pancreas. The rabbits receiving insulin-loaded Carbopol gels exhibited a monophasic decrease in serum glucose concentrations. The glucose levels reached the nadir (58.62% of the baseline value) in 2 h and the hypoglycaemic effect lasted for 24 h. Both the gels containing Tween 80 and sodium
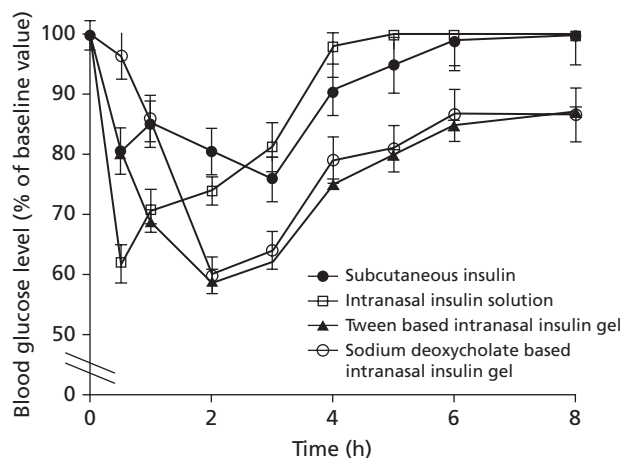
deoxycholate decreased the plasma glucose concentrations to almost the same level and duration of action, but gels with the former enhancer recorded a significantly higher $AAC_{(0-24)}$ value. The difference in the $AAC_{(0-24)}$ values was prominent only in the first 2 h. The significantly higher $AAC_{(0-24)}$ value observed for the gels compared with the insulin solution indicated a more sustainable hypoglycaemic effect for the gels (Table 2).

Discussion

In the preliminary work, Carbopol gels were prepared by neutralization with alkali to permit the ionization of carboxylic groups of Carbopol forming strong gels. However, insulin did not dissolve in these neutralized gels and remained dispersed, resulting in non-transparent aqueous gels. Therefore, preparation of insulin gels in this study was performed by the homogenization technique. The levels of the four formulation factors examined in this study were selected based on preliminary investigations. Tween 80 was selected as one of the permeation enhancers for this study because of the better permeation enhancement it showed compared with β-cyclodextrin and Myrj 53. In addition, Tween 80 has been reported in the literature to be a suitable enhancer for high molecular weight hydrophilic peptides such as insulin.[4]

All the twenty four gel formulations prepared and evaluated in this study exhibited pseudoplastic flow. At any particular rate of shear, increasing the molecular weight of PEG or the Carbopol concentration resulted in an expected increase in gel viscosity. Moreover, higher viscosities were obtained upon preparing the formulations under cold conditions, which is in contrast to Bonacucina *et al.*[12] where an increase in the viscosity of gels prepared with PEG and Carbopol 974 or Carbopol 971 was obtained when heated to 70°C. The drop in viscosity with the application of heat observed in this study might be due to the increase in the kinetic energy of the system, which resulted in a weaker gel network structure with a lower resistance to flow. The use of sodium deoxycholate as permeation enhancer imparted higher viscosity compared with Tween 80, which might be due to the viscosity inducing property of sodium deoxycholate.

The increase in the prepared gel viscosity with increasing the PEG molecular weight or Carbopol concentration or changing the method of preparation was reflected in the release of insulin from the gels, where more retardation was observed. Studying release kinetics showed that diffusion through the gel

Figure 5 In-vivo evaluation of insulin formulations. Blood glucose levels of different insulin formulations in rabbits ($n = 6$).

Table 2 In-vivo evaluation of insulin formulations

Insulin formulation	$AAC_{(0-24)}$ (μg/ml)	C_{max} (μg h/ml)	t_{max} (h)
Subcutaneous insulin	12.043 ± 1.03	24.08 ± 0.66	3 ± 0.32
Intranasal solution	13.091 ± 0.91	38.21 ± 0.49	0.5 ± 0.58
Tween based intranasal gel	51.725 ± 1.36	41.3 ± 0.89	2 ± 0.32
Sodium deoxycholate based intranasal gel	45.724 ± 1.73	40.0 ± 0.96	2 ± 0.44

Comparison of the area above the blood glucose level–time curve for 24 h (AAC_{0-24}), the maximum percent decrease in blood glucose levels (C_{max}) and the time required to reach that level (t_{max}) between rabbits receiving the different insulin formulations ($n = 6$).

had a great influence on the insulin release from most of the gel formulations, except for those formulations containing PEG with the higher molecular weight and the highest concentration of Carbopol, which were dominantly controlled by the swelling and relaxation of the matrix.

The optimal gel formulations, which were selected based on the results of the factorial design, were the gels containing PEG 400 as the gelation medium together with 4% Carbopol and prepared under hot conditions. These gels recorded acceptable viscosity and release profiles (100% insulin release within 8 h) in the presence of any of the two permeation enhancers used.

In-vivo, these gels succeeded in inducing monophasic more sustainable hypoglycaemic effects (24 h) in rabbits compared with subcutaneous insulin and intranasal insulin solution. The brief resident time of the solution in the nasal cavity resulted in a short period of hypoglycaemic effect that lasted for only 4 h. Therefore, its potential might only be considered when it is used as an adjunct to subcutaneous treatment.[15] The higher extent of hypoglycaemia observed for the gels might be due to the absorption of water from the gel base (water influx from the gel into the body), which facilitated the movement of insulin through the intercellular channels. The bioadhesive property of Carbopol might also increase the residence time of the gel in the nasal mucosa and its interaction with the mucosal calcium ions, which would result in opening of the mucosa intercellular tight junctions.[16] Moreover, the permeation enhancers used in the gels (sodium deoxycholate or Tween 80) have been reported to reversibly increase the transepithelial conductance across the nasal membrane and produce high membrane concentrations of insulin monomers via solubilization in mixed micelles.[17] They also form reverse micelles within the nasal membranes, through which insulin monomers can diffuse through polar channels into the bloodstream. In addition, they had the ability to inhibit the enzymatic degradation in the nasal mucosa and decrease the viscosity and elasticity of the mucus, which might also facilitate penetration of insulin through the mucus layer.[17,18] The relatively more viscous sodium deoxycholate containing gel might be the reason for the slower hypoglycaemic effect for this gel compared with Tween 80 within the first 2 h after administration. This effect might also be due to a slower action of sodium deoxycholate as an enhancer than Tween 80 in opening the intracellular tight junctions.

Conclusions

This study showed the feasibility of preparing clear transparent Carpobol gels in hydrophilic co-solvents by homogenization rather than neutralization. Optimization of the gel formulation composition such as the type and molecular weight of the gelation medium, the Carbopol concentration along with the type of permeation enhancer used and preparation conditions were imperative for the development of gels with suitable viscosity and release profiles. This would prolong the gel residence time in the nasal cavity and consequently produce a more sustainable hypoglycaemic effect than the intranasal solution. Furthermore, the intranasal insulin gel would be more convenient for patients than subcutaneous injection.

Declarations

Conflict of interest

Funding

References

1. Home PD *et al*. Alternative routes and methods of insulin delivery. *Neth J Med* 1985; 28: 32–36.
2. Wang B *et al*. The role of CD8+ T cells in the initiation of insulin-dependent diabetes mellitus. *Eur J Immunol* 1996; 26: 1762–1769.
3. Hinchcliffe M, Illum L. Intranasal insulin delivery and therapy. *Adv Drug Deliv Rev* 1999; 35: 199–234.
4. Craft S *et al*. Therapeutic effects of daily intranasal insulin administration in early Alzheimer's disease. *Alzheimers Dement* 2006; 2: S63.
5. Merkus FWHM *et al*. The influence of absorption enhancers on intranasal insulin, absorption in normal and diabetic subjects. *J Control Release* 1996; 41: 69–75.
6. Luppi B *et al*. Novel mucoadhesive nasal inserts based on chitosan/hyaluronate polyelectrolyte complexes for peptide and protein delivery. *J Pharm Pharmacol* 2009; 61: 151–157.
7. Wang J *et al*. Aminated gelatin microspheres as a nasal delivery system for peptide drugs: evaluation of in vitro release and in vivo insulin absorption in rats. *J Control Release* 2006; 113: 31–37.
8. Zhang X *et al*. Nasal absorption enhancement of insulin using PEG-grafted chitosan nanoparticles. *Eur J Pharm Biopharm* 2008; 68: 526–534.
9. Kashyap N *et al*. Design and evaluation of biodegradable, biosensitive *in-situ* gelling system for pulsatile delivery of insulin. *Biomaterials* 2007; 28: 2051–2060.
10. D'Souza R *et al*. Insulin gel as an alternate to parenteral insulin: formulation, preclinical, and clinical studies. *AAPS PharmSciTech* 2005; 6: E184–E189.
11. Gordon GS *et al*. Nasal absorption of insulin: enhancement by hydrophobic bile salts. *Proc Natl Acad Sci* 1985; 82: 7419–7423.
12. Bonacucina G *et al*. Rheological, mucoadhesive and release properties of Carbopol gels in hydrophilic cosolvents. *Int J Pharm* 2004; 282: 115–130.
13. Khan KA. The concept of dissolution efficiency. *J Pharm Pharmacol* 1975; 27: 48–49.
14. Gohel MC, Panchal MK. Novel use of similarity factors f2 and S_d for the development of diltiazem HCl modified-release tablets using a 3(2) factorial design. *Drug Dev Ind Pharm* 2002; 28: 77–87.
15. Touitou E, Rubinstein A. Targeted enteral delivery of insulin to rats. *Int J Pharm* 1986; 30: 95–99.
16. Li Y, Mitra AK. Effects of phospholipid chain length, concentration, charge, and vesicle size on pulmonary insulin absorption. *Pharm Res* 1996; 13: 76–79.
17. Moses AC *et al*. Insulin administered intranasally as an insulin-bile salt aerosol. Effectiveness and reproducibility in normal and diabetic subjects. *Diabetes* 1983; 32: 1040–1047.
18. Nolte MS *et al*. Biological activity of nasally administered insulin in normal subjects. *Horm Metab Res* 1990; 22: 170–174.