Nasal Insulin Delivery with Dimethyl-β-Cyclodextrin as an Absorption Enhancer in Rabbits: Powder More Effective than Liquid Formulations

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The nasal absorption of insulin using dimethyl-β-cyclodextrin (DMβCD) as an absorption enhancer in rabbits was studied. The nasal administration of insulin/DMβCD liquid formulations did not result in significant changes in serum insulin and blood glucose concentrations. In contrast, previous experiments in rats showed that the addition of DMβCD to the liquid nasal formulation resulted in an almost-complete insulin absorption, with a concomitant strong hypoglycaemic response. Apparently, the effect of the cyclodextrin derivative on insulin absorption differs between animal species following nasal delivery of insulin/DMβCD solutions. On the other hand, nasal administration of the lyophilized insulin/DMβCD powder dosage form in rabbits resulted in increased serum insulin concentrations, and a maximum decrease in blood glucose of about 50%. The absolute bioavailability of the nasally administered insulin/DMβCD powder was 13 ± 4%, compared to 1 ± 1% for both an insulin/DMβCD liquid and an insulin/lactose powder formulation. It is concluded that insulin powder formulations with DMβCD as an absorption enhancer are much more effective than liquid formulations.

KEY WORDS: insulin; nasal delivery; dimethyl-β-cyclodextrin; rabbit; interspecies differences; powder dosage form.

INTRODUCTION

Poor absorption of large hydrophilic peptide and protein drugs such as insulin is a major limitation in the nasal drug delivery of these compounds (1). The absorption may be improved by coadministration of absorption enhancers. Many compounds, as, for example, bile salts, fusidates, fatty acids, and cyclodextrins (2), have been investigated as potential absorption enhancers. Although there are various agents effective in improving the nasal absorption of insulin, problems encountered with most absorption enhancers are possible irritation, damage, and ciliotoxic effects on the nasal epithelial membrane (3,4). In previous studies from our laboratory it was demonstrated that addition of dimethyl-β-cyclodextrin (DMβCD) to liquid nasal preparations largely improved the absorption of insulin in rats, with concomitant strong decreases in blood glucose concentrations (2,5). The insulin bioavailability after administration of insulin with DMβCD was about 100%. The effect of DMβCD on ciliary movement, on the other hand, was concentration dependent and reversible. The ciliotoxicity was particularly mild when compared with other commonly used nasal absorption promoters (6). DMβCD, therefore, seems to be a safe and effective enhancer for use in nasal insulin therapy. Pilot experiments with volunteers, however, revealed that liquid insulin/DMβCD formulations were not effective in enhancing nasal insulin absorption in man. In this pilot study nasal sprays of 20 to 80 IU with 5 or 20% (w/v) DMβCD were administered to healthy subjects who had fasted overnight. None of the subjects showed significant changes in blood glucose or serum insulin concentrations (unpublished results). Apparently, large interspecies differences in the absorption of insulin with DMβCD exist (7). In order to examine these species differences more extensively, in the present study the intranasal absorption of insulin with DMβCD in rabbits was investigated. Hereto, solutions containing different amounts of insulin and DMβCD were employed. The effect of powder versus liquid formulations on nasal insulin absorption in rabbits was also investigated.

MATERIALS AND METHODS

Materials

Human insulin powder (20 IU/mg) was obtained from Diosynth (Oss, The Netherlands). Dimethyl-β-cyclodextrin (DMβCD) was from Avebe (Foxhol, The Netherlands). Sodium taurodihydrofusidate (STDHF) and aprotinin (10 kIU/ml) were gifts from CalBio (Mountain View, CA) and Bayer (Leverkusen, Germany), respectively. Bacitracin and *N*-acetyl-L-cysteine were obtained from Sigma (St. Louis, MO) and Hypnorm from Janssen (Beerse, Belgium).

Preparation of Nasal Dosage Forms

Nasal insulin solutions were prepared by dissolving human insulin powder in 2.5 m*M* HCl in 0.9% saline. The solutions were adjusted to a pH value of 5 with 0.1 *M* NaOH. Final insulin concentrations were 2 and 4 mg/mL. The absorption enhancers, at concentrations as indicated in Table I, were subsequently added to the insulin solutions. Placebo formulations contained 5% (w/v) DMβCD in 0.9% saline. For intravenous administration an insulin solution of 0.5 mg/mL in saline without DMβCD was used. Powder insulin preparations were made by dissolving human insulin in 2.5 m*M* HCl to a concentration of 4 mg/mL. DMβCD or lactose was subsequently added to a molar concentration of 37.5 m*M* [5% (w/v) DMβCD, 1.35% (w/v) lactose]. The insulin solutions were then frozen in liquid N_2 and lyophilized. Lyophilized DMβCD was used as a control. The powder preparations were stored at 4°C until use. The amount of insulin in all formulations was checked prior to the experiments by means of reversed-phase HPLC analysis. A Chromsep C18 column was used. The mobile phase was isocratic with a buffer to acetonitrile ratio of 76:24. The buffer consisted of 0.1 *M* KH_2PO_4 with 1% (v/v) triethyl amine adjusted to pH 3 with phosphoric acid.

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Table I. Administration of Nasal Insulin Solutions to Rabbits[a]

Dose (IU/rabbit)	Additive	AUC insulin (μU/mL × min)	F (% dose)	n
0	DMβCD, 5%	637 ± 269	0	6
2	None	929 ± 659	1.3 ± 3.2	6
2	DMβCD, 5%	798 ± 201	0.8 ± 1.0	6
2	DMβCD, 30%	1279 ± 835	3.2 ± 4.2	3
2	STDHF, 1%	1187 ± 560	2.8 ± 3.0	4
2	DMβCD, 5%, + STDHF, 1%	502 ± 53	0	3
2	DMβCD, 5%, + bacitracin, 10%	1196	2.8	2
2	DMβCD, 5%, + aprotinin, 0.5%	665	0.1	2
2	DMβCD, 5%, + N-Ac.Cys., 10%	1146	2.6	2

[a] Data are presented as the mean ± SD, except when only two animals were used (the mean is given in that case). AUC values have been calculated up to 3 hr after insulin administration. F represents the insulin bioavailability determined up to 3 hr. N-Ac.Cys., N-acetyl-L-cysteine.

Animals

Two groups of six New Zealand White female rabbits were used. They were purchased from Iffa Credo Broekman (Someren, The Netherlands). The first group of rabbits had an average weight of 3.5 ± 0.2 kg (mean ± SD) at the beginning and 3.6 ± 0.2 at the end of the study, whereas the second group had an average weight of 1.9 ± 0.1 kg at the start and 2.7 ± 0.2 at the end of the study period. The animals were kept in stainless-steel cages and were fed a commercial laboratory diet.

Absorption Studies

In a first series of experiments the effect of DMβCD in liquid formulations on nasal insulin absorption in rabbits was studied. The rabbits were fasted overnight (±16 hr), while they had free access to drinking water. The rabbits were sedated by subcutaneous injections of 0.5 mL/kg Hypnorm; additional injections of 0.25 mL/kg were given after 2.5 hr. Nasal insulin solutions were administered using an Hamilton microliter syringe attached with 5-cm PE tubing. The tubing was inserted 1.0 to 1.5 cm into a naris, and 50 μL drug solution, corresponding to 2 IU insulin, was instilled. A 2-IU insulin intravenous bolus injection (0.2 mL of an 0.5 mg/mL insulin solution in saline) and intranasal placebo containing 5% DMβCD in saline were given to determine the absolute insulin bioavailability of the nasal formulations. The insulin preparations were given in a random order to each of the six rabbits. A washout period of 1 week was kept between subsequent administrations. Nasal formulations of insulin with DMβCD and a second absorption promoter [N-acetyl-L-cysteine (10%, w/v), aprotinin (0.5%, v/v), bacitracin (10%, w/v), or STDHF (1%, w/v)] were given to some of the rabbits to screen for additional enhancement of insulin absorption.

In a second series of experiments intranasal administration of insulin powder versus insulin liquid formulations was studied. The dose administered was increased from 2 IU/rabbit (approx. 0.55 IU/kg) to 4 IU/rabbit (approx. 1.7 IU/kg). The insulin solutions were administered as described above. A volume of 50 μL of 4 mg/mL insulin with 5% (w/v) DMβCD was instilled. Powder formulations were administered using an insufflator as shown in Fig. 1. PE tubing containing the powder formulation was attached to a valve which was placed on a 10-mL syringe with compressed air. The tubing was inserted 1 to 1.5 cm into one of the nares, and powder was propelled into the nose by opening the valve. The tubing was weighed before and after powder administration to calculate the exact insulin dose administered. This was 4.0 ± 0.4 and 5.0 ± 2.4 IU (mean ± SD) insulin and 1.9 ± 0.2 and 2.4 ± 1.1 μmol additive for the insulin/DMβCD and insulin/lactose powder formulations, respectively. In addition, an intravenous bolus injection of 2 IU insulin as well as 2.0 ± 0.2 μmol (mean ± SD) intranasal lyophilized DMβCD were given to the rabbits. The insulin preparations were given in a random order with a washout period of 1 week.

Venous blood samples were taken from an ear vein at regular time intervals. They were allowed to clot and were then centrifuged to obtain serum. The serum samples were stored at −20°C until analysis.

Analytical Procedures

Blood glucose was measured immediately after blood sampling with Haemo-glukotest sticks and a Reflolux II Reflectance meter (Boehringer Mannheim, Germany). Serum insulin concentrations were determined using radioimmunoassay (Insulin R-100, Pharmacia, Woerden, The Netherlands). The detection limit of the assay was below 2 μU/mL. The antiserum showed 100% cross-reactivity with bovine and porcine insulin and 0.2% with C peptide.

Data Analysis

Blood glucose values were calculated as percentage of the blood glucose concentrations measured just before the

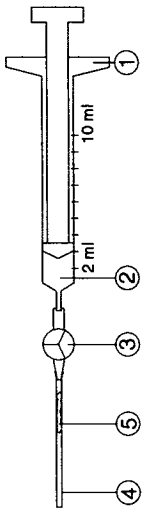

Fig. 1. Schematic representation of the device used for the nasal administration of powder dosage forms. 1, Airtight syringe; 2, chamber with compressed air; 3, valve; 4, polyethylene tubing; 5, powder dosage form. The device was adapted from the powder administration device described by Sörensen (22).

start of the experiment. The areas under the individual serum insulin and blood glucose versus time curves (AUC) were calculated using the linear trapezoidal rule. Bioavailabilities were calculated using the individual insulin AUC data according to the formula $(AUC_{IN} - AUC_{plac})/(AUC_{IV} - AUC_{plac}) \times Dose_{IV}/Dose_{IN} \times 100\%$.

Statistical Evaluation

For statistical evaluation a one-way analysis of variance test and a Student's t test for paired results were used. Differences were assigned to be significant for values of $P < 0.05$.

RESULTS

The basal serum insulin levels of the rabbits as measured after placebo administration varied between 1 and 15 μU/mL. The blood glucose concentration was 7.7 ± 1.0 mmol/L at the start and showed a small increase during the 3 hr of the experiment (Fig. 2).

The results of the first nasal absorption studies with insulin solutions containing DMβCD are presented in Table I. Intranasal administration of insulin (2 IU/rabbit) solutions without additives or with 5% (w/v) DMβCD did not result in increased serum insulin levels as compared to the serum insulin concentrations after placebo administration. Also blood glucose concentrations were not different from placebo administration. The insulin bioavailability was less than 1% following nasal delivery of both the insulin solutions without additive and with 5% (w/v) DMβCD. Increasing the concentration of the cyclodextrin derivative to as much as 30% (w/v) in the nasal formulation did not significantly improve the insulin absorption. Coadministration of the proteolytic enzyme inhibitors aprotinin and bacitracin or the

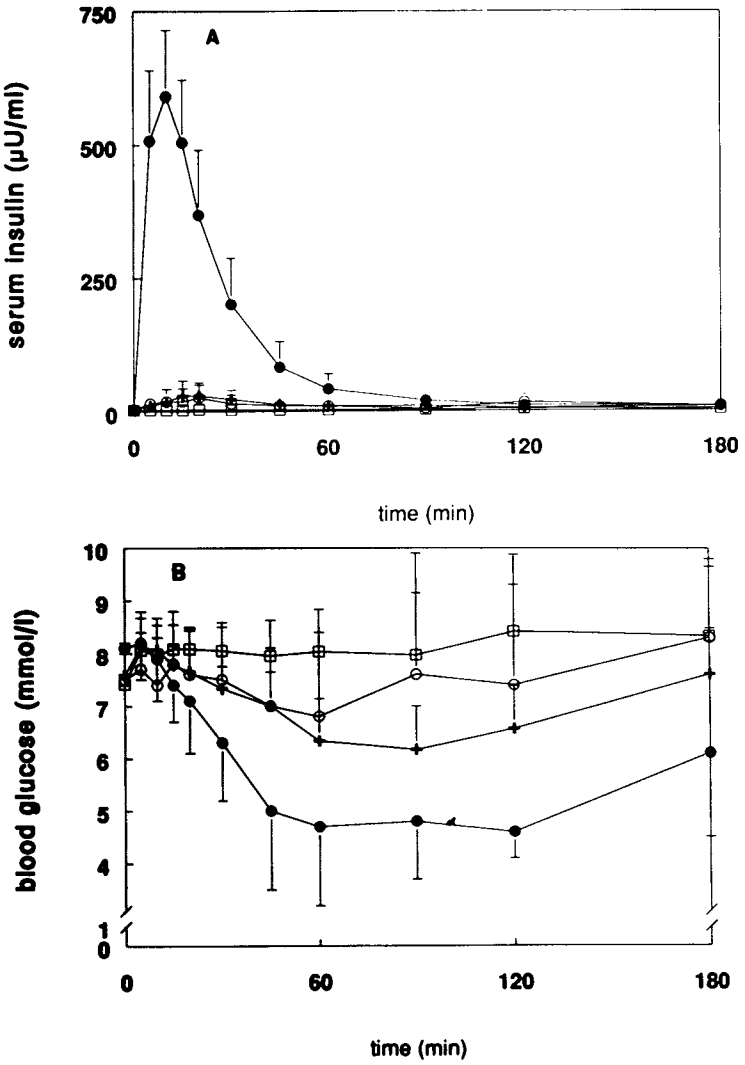

Fig. 2. Mean serum insulin concentrations (A) and mean blood glucose concentrations (B) following intranasal administration of (□) placebo; (○) insulin/DMβCD solution; (+) insulin/lactose powder; (●) insulin/DMβCD powder. Error bars indicate the SD of six animals.

mucolytic agent *N*-acetyl-L-cysteine to the insulin/DMβCD solutions did not have an additional effect on insulin or glucose levels (Table I). Nasal administration of insulin with STDHF and DMβCD resulted in an insulin bioavailability that was not different from zero, whereas addition of STDHF without DMβCD resulted in a slightly improved insulin absorption, but the results were not significantly different from administration of insulin without any absorption enhancing compound.

In a second series of experiments the nasal insulin absorption was studied following delivery of an insulin/DMβCD powder formulation in another group of six rabbits. The insulin dose was increased to 4 IU per animal. Serum insulin and blood glucose versus time curves are shown in Fig. 2, and the pharmacokinetic data are presented in Table II. Nasal administration of a powder formulation containing 4 IU lyophilized insulin and DMβCD resulted in remarkably increased serum insulin levels compared to placebo administration (powder formulation of only DMβCD). The time to reach the peak serum concentration of insulin was about 10 min, indicating that the insulin dose was quickly absorbed. The peak serum concentration was 640 ± 104 μU/mL. The insulin concentrations returned to endogenous levels after about 120 min. A decrease in blood glucose of 50% to a minimum of 4 ± 0.8 mmol/L was observed, and after the 3 hr of the experiment the blood glucose concentrations were still reduced. A very small increase in serum insulin levels was obtained when the same amount of insulin and DMβCD was administered as a nasal liquid formulation. Moreover, a powder formulation containing 4 IU insulin and lactose also had minor effects on serum insulin and blood glucose concentrations. A maximum decrease in blood glucose of about 20% of its initial value was observed. The nasal insulin bioavailability, corrected for the actual dose administered, was 13 ± 4% for the insulin/DMβCD powder formulation and negligible (1 ± 1%) for both the insulin/lactose powder and the insulin/DMβCD liquid formulations.

DISCUSSION

A number of animal models have been described in the literature to study the absorption of drugs across the nasal mucosa, including the anesthetized rat model developed by Hirai *et al.* (8). Rabbits, dogs, sheep, monkeys, and mice are also used to investigate nasal drug absorption *in vivo* (9). In order to be able to extrapolate experimental results from an animal species to man, the choice of the model is important. We demonstrated that DMβCD improved the nasal absorption of insulin in rats to a large extent (5). In contrast, DMβCD in solution did not have an effect on insulin absorption in rabbits, as shown in the present study. Similarly, DMβCD in solution did not affect insulin absorption in man (unpublished observations), suggesting that the rabbit may be a better animal model to study nasal insulin absorption. This, however, does not hold in general when studying nasal peptide delivery. Although it is difficult to compare studies using different animal models because of variations in experimental conditions and nasal formulations, it has been shown that nasal peptide absorption in different animal models varies considerably, depending on both peptide and absorption enhancer under study (2,6,10). Interspecies differences in the nasal absorption of drugs may be accounted for by both physiological (such as mucus flow, mucus constituents, and enzyme activity) and anatomical (such as nasal volume, nasal length, and conchae structure) factors (9,11). The anatomical differences between species will affect the deposition and subsequent spreading and dilution of liquid drug formulations in the nasal cavity. For powder formulations these effects will probably be less pronounced, since powders spread less easily and dissolve at the area of disposition.

The present study demonstrates that a remarkable improvement of nasal insulin absorption in rabbits can be obtained when an insulin/DMβCD powder instead of a liquid dosage form is administered. Although the same amounts of insulin and DMβCD were given using the liquid and powder preparations, the mean insulin bioavailability of the powder formulation was 13 versus 1% for the liquid formulation. The improved insulin absorption from the powder formulation may be accounted for by a different deposition pattern in the nose which might influence drug absorption (12). It may also be possible that the dissolution of the powder dosage form in the nasal mucosa will attract water from the nasal epithelium, resulting in a widening of the spaces between tight junctions and thus facilitating drug absorption (13). However, the osmotic effect of the powder will probably not be a major mechanism for increased insulin absorption, since insufflation of an equimolar amount of lactose did not improve insulin absorption compared to administration of the insulin/DMβCD saline solution. DMβCD has been suggested to act upon the nasal epithelium via association with membrane lipids. In addition, DMβCD has been shown to inhibit pro-

Table II. Administration of Nasal Insulin Powders and Solutions to Rabbits[a]

Dose (IU/rabbit)	Formulation (μmol additive/dose)	T_{max} (min)	C_{max} (μU/mL)	AUC glucose (% × min)	AUC insulin (μU/mL × min)	F (% dose)
0	DMβCD powder, 2.0 ± 0.2			19,906 ± 2,192	703 ± 276	0
4	DMβCD solution, 1.9	25 ± 16	29 ± 27	18,275 ± 4,018	2,184 ± 1,016**	1.1 ± 0.9
4 ± 0.4	DMβCD powder, 1.9 ± 0.2	9.2 ± 3.8	640 ± 104*	12,268 ± 2,006***	16,838 ± 4,272***	12.9 ± 4.4*
5 ± 2.4	Lactose powder, 2.4 ± 1.1	23 ± 11	35 ± 25	16,461 ± 1,350***	1,999 ± 604***	1.1 ± 0.8

[a] Data are presented as the mean ± SD of six animals. T_{max} represents the time at which the insulin peak concentration was reached. C_{max} is the insulin peak concentration. AUC values have been calculated up to 3 hr after insulin administration. F represents the insulin bioavailability determined up to 3 hr.

* Significantly different from the insulin/DMβCD solution and the insulin/lactose powder, $P < 0.01$.

** Significantly different from DMβCD powder (placebo), $P < 0.05$.

*** Significantly different from DMβCD powder (placebo), $P < 0.01$.

teolytic enzyme activity and to decrease the aggregation of insulin into hexamers (14). Dissolution of the insulin/DMβCD powder in the nasal mucosa will result in a relatively high local concentration of insulin and DMβCD, thereby favoring the absorption of insulin. The dissolution of the powder in the hydrated mucus layer is most likely very rapid, since the insulin is quickly absorbed as apparent from the short time necessary to reach peak serum insulin concentrations.

Many reports have been published on nasal delivery of peptide and protein drugs using powder formulations (15–18). The absorption from these powder formulations either with or without absorption enhancer was mostly very effective. Nevertheless, in the present study the difference in absorption from the powder versus the liquid formulation containing a similar amount of insulin and DMβCD was unexpectedly large. Powders of biodegradable starch microspheres increased the absorption of insulin in rats and sheep (19,20). This enhanced absorption may be due to a prolonged residence time in the nose (21), but the microspheres probably also facilitate the permeation of insulin across the nasal epithelial cells by widening the spaces between the tight junctions (13). The bioerodible powders are able to absorb water to a large extent but are not water soluble, in contrast to the powder used in this experiment which dissolves very well.

Powder dosage forms of peptide and protein drugs can have advantages over liquid formulations. For instance, in powders the chemical stability of the drug is increased, a preservative in the formulation is not required, and it is possible to administer larger amounts of drug and excipients (17).

In conclusion, the present study demonstrates that nasal administration of a solution of insulin with 5% DMβCD to rabbits does not result in improved insulin absorption as compared to administration of insulin without any excipient. Considering the previously observed beneficial effect of DMβCD solutions on nasal insulin absorption in rats (5), substantial interspecies differences exist in the absorption enhancing effect of this cyclodextrin derivative (7). On the other hand, insulin/DMβCD powder formulations appear to result in a large improvement of nasal insulin absorption in rabbits, as evident from increased serum insulin and decreased blood glucose concentrations. The observed differences in efficacy of nasal insulin absorption in rabbits from liquid and powder insulin/DMβCD dosage forms may be important for nasal insulin delivery. Nevertheless, the effectiveness and the safety of the powder formulation in man remain to be established.

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