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International Journal of Pharmaceutics 250 (2003) 415–422
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Influence of multiple nasal administrations of bioadhesive powders on the insulin bioavailability

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Received 8 May 2002; received in revised form 28 August 2002; accepted 8 October 2002

Abstract

Peptides and more especially insulin are mainly used in therapies that need multiple drug administration. As peptides are highly potent, it is required that their bioavailability remains constant even during a long term administration. In this study, the bioavailability and blood glucose levels are reported after multiple nasal administration of insulin via two bioadhesive platforms consisting of a cospray dried mixture of Amioca® starch and Carbopol® 974P (1/3) and a physical mixture of drum dried waxy maize starch and Carbopol® 974P (9/1), respectively. The experiments were performed in rabbits and the formulations were administered during 8 consecutive days. The bioavailability and the maximal decrease of the blood glucose level were determined on the first and last day of the insulin administration. These two parameters were decreased on the eighth day compared with the first day of administration. When the formulations were not administered from day 2 until day 7, the bioavailability on the eighth day compared with the first day of administration was not modified. It was concluded that daily administrations of the bioadhesive formulations affected the nasal bioavailability of insulin in rabbits.

Keywords: Multiple administration; Bioavailability; Nasal drug delivery; Insulin; Bioadhesion

1. Introduction

The use of transnasal administration of drugs has gained interest in recent years for the systemic delivery of therapeutically active peptides. Due to the hydrophilic properties and high molecular weight of peptides, their transnasal absorption is rather low. Different strategies were proposed to improve the bioavailability through the nasal route such as, the use of absorption enhancers, enzyme inhibitors, microspheres and bioadhesives. Callens and Remon (2000) improved the nasal absorption of insulin in rabbits using a bioadhesive powder formulation containing drum dried waxy maize starch (mainly amylopectin) and Carbopol® 974 P and obtained a bioavailability of 14%. As peptides are mostly used in chronic therapy the irritation potency of the formulation is a major concern. The powder formulation described by Callens et al. was evaluated for its

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0378-5173/02/$ - see front matter
PII: S0378-5173(02)00555-0

irritation potency on nasal rabbit and slug mucosa (Callens et al., 2001). The authors concluded that the effect of the powder on the mucosa was negligible and that it was possibly safe for long term treatment. No information is available in the literature about the consequences of multiple administration of a powder formulation on the bioavailability of peptides. Although the maintenance of a constant bioavailability and therapeutic effect of the peptides during a long therapy is of major importance due to their high potency. The effect of multiple administration has only been investigated in terms of toxicity namely the effect on the morphology (Hjortkjaer et al., 1999; Ugwoke et al., 2000a), the release of enzymes from the epithelium (Pujara et al., 1995), the mucociliary clearance (Holmberg et al., 1994) and the ciliary beat frequency (Ugwoke et al., 2000a). This study investigated the influence of eight daily administrations of two powder formulations to rabbits on the bioavailability and therapeutic effect of the peptide insulin. The first powder formulation consisted of a co-spray dried mixture of Amioca® starch and Carbopol® 974 P (1/3) and the second one being a physical mixture of drum dried waxy maize starch and Carbopol® 974 P (9/1).

2. Materials and methods

2.1. Materials

Actrapid® HM 100 (100 IU/ml) (human monocomponent insulin) was obtained from Novo-Nordisk (Bagsvaerd, Denmark).

The co-spray dried mixture of Amioca® starch and Carbopol® 974P (1/3) was received from National Starch and Chemical Company (Bridgewater, USA), drum dried waxy maize starch from Eridania Béghin-Say, Cerestar (Vilvoorde, Belgium) and Carbopol® 974P from BF Goodrich Co. (Cleveland, OH, USA).

2.2. Preparation of the insulin formulations

Dispersions were prepared by adding, respectively, 10 and 30 ml distilled water to 1 g of the co-spray dried mixture of Amioca® starch and Carbopol® 974P (1/3) or of the physical powder mixture containing drum dried waxy maize starch and Carbopol® 974P (9/1). After neutralization of the dispersion with NaOH 2 M till pH 7.4, the insulin solution (Actrapid® HM 100) was added in order to obtain ratios of 1 IU of insulin per mg powder. The dispersions were lyophilized in vials using an Amsco-Finn Aqua GT4 freeze-dryer and next passed through a sieve of 63 μm. The powder fraction below 63 μm, with less than 2% of the powder particles having a diameter lower than 10 μm (analyzed via laser diffraction), was stored in a desiccator at 4–8 °C until use. Powder formulations without insulin were also prepared as mentioned above but without addition of the insulin solution.

2.3. Insulin formulations for i.v. administration

An insulin solution of 0.8 IU/ml was prepared by diluting the Actrapid® HM 100 in a phosphate buffer pH 7.4, next 0.5 ml was administered intravenously.

2.4. Animal experiment

New Zealand white rabbits weighing 3 ± 0.3 kg were used. The animals were fasted 16 h prior to an insulin administration, with free access to water and next sedated by an IM injection of 0.15 ml/kg Thalamonal® (Janssen Pharmaceutica, Beerse, Belgium). Sedation was maintained during 3 h with additional doses of 0.075 ml/kg. The animals were not anaesthetized during the experiment. The rabbits received 0.4 IU insulin during the intravenous administration.

Three non-cross-over experiments were performed on six to eight rabbits to determine the influence of multiple nasal administrations. An overview of the experiments is given in Table 1. In the first experiment, powder formulations were administered daily during 8 days. Only on the first day and the last day, insulin was added to the formulations, and insulin bioavailability and blood glucose levels decrease were determined. In the second experiment insulin containing powder was administered on the first and eighth day of the

Table 1
Overview of the administration of the co-spray dried mixture of Amioca® starch and Carbopol® 974P (1/3) (Amioca/Carb. 1/3) and the physical mixture of drum dried waxy maize starch and Carbopol® 974P (9/1) (DDWM/Carb. 9/1) and insulin (8.7 IU per nostril) during the three 8-day experiments in rabbits ($n = 6–8$)

Day 1	Day 2–7	Day 8
Experiment 1		
DDWM/Carb. 9/1 + insulin	DDWM/Carb. 9/1	DDWM/Carb. 9/1 + insulin
Amioca/Carb. 1/3 + insulin	Amioca/Carb. 1/3	Amioca/Carb. 1/3 + insulin
Experiment 2		
Amioca/Carb. 1/3 + insulin	–	Amioca/Carb. 1/3 + insulin
Experiment 3		
Amioca/Carb. 1/3	Amioca/Carb. 1/3	Amioca/Carb. 1/3 + insulin

experiment. From day 2 till day 7 no powder was administered. The third experiment was done like the first one, while no insulin was added to the formulation on the first day of the experiment. The powder formulations were administered intranasally in both nostrils using polyethylene tubes (Medisize N.V., Hillegom, The Netherlands) filled with 10 mg powder containing approximately 8.7 IU insulin after lyophilization. The tubes were filled the evening before administration under conditions of low relative humidity (20%) and ambient temperature. The powder was released from the tubes using a syringe containing 1 ml compressed air. This device was based on a system developed by Sørensen (1991). Blood samples were collected from the ear veins, when using insulin containing powder, at −5, 2, 5, 10, 15, 20, 25, 30, 35, 45, 60, 90, 120, 150 and 180 min after intranasal administration and at −5, 1, 5, 10, 15, 20, 30, 40, 50 and 60 min after the intravenous administration. Blood glucose levels were measured immediately after blood collection with a Glucotouch meter using Glucotouch test strips (Lifescan Benelux, Beerse, Belgium). The sera were separated by centrifugation ($700 \times g$, 5 min) and the samples were stored frozen at −20 °C until RIA analysis was done in duplicate (Coat-A-Count® kit, DPC, Humbeek, Belgium). The individual serum concentration–time profiles were analysed by MW/Pharm version 3.0 (Mediware, 1987–1991, Utrecht, The Netherlands) and the maximal serum insulin concentrations (C_{max}) were determined from the individual serum concentration–time profiles. Statistical significance was calculated according to One-way ANOVA ($P < 0.05$) followed by a Scheffé test.

2.5. Estimation of the viscosity

Viscosity determination of the powder dispersions was performed because of the possible influence of the viscosity on the nasal mucociliary clearance. A dispersion was prepared, conform the method used by Ceulemans and Ludwig (2002), containing 8% (g/v) mucin (Mucin type II: Crude from Porcine Stomach, Sigma–Aldrich Chemie, Steinheim, Germany) in simulating nasal fluid composed of 7.45 mg/ml NaCl, 1.29 mg/ml KCl and 0.315 mg/ml $CaCl_2$ (Melon, 1968). A 10% (w/v) dispersion of the co-spray dried mixture of Amioca® starch and Carbopol® 974P (1/3) or of the mixture of drum dried waxy maize starch and Carbopol® 974 P (9/1) was prepared in the above mentioned mucin dispersion. The choice for using a 10% dispersion can be supported by considering that 10 mg administered powder is dispersed in approximately 100 μl nasal fluid (Gizurarson, 1993a).

G'' (viscosity) was determined with a TA Instruments CSL^2 Rheometer (Leatherhead, UK) applying an oscillation stress of 1.4 Pa and using a cylinder with radii R_1, 20; R_2, 20.38; R_3, 21.96 and R_4, 22.38 for the mucus dispersion and a cone of 4 cm with an angle of 1.59° for the dispersion of mucin and bioadhesive powder.

2.6. Determination of the mucociliary clearance by gamma scintigraphy

2.6.1. Radiolabelling procedure

The procedure was based on the method used by Ridley et al. (1995), Soane et al. (1999). DDWM/Carbopol® 974 P (9/1) (200 mg) was dispersed in 800 μl $SnCl_2$, 1 ml sodium pertechnetate containing about 40 mCi of activity and 38.2 ml distilled water. The dispersion was left under continuous

stirring for 10 min and was then centrifuged at 3000 rpm for 20 min. The supernatant was removed and the dispersion washed with 20 ml distilled water and centrifuged again. The dispersion was lyophilized in vials using an Amsco-Finn Aqua GT4 freeze-dryer and next passed through a sieve of 63 μm. Less than 2% sodium pertechnetate remained unbounded after lyophilization.

2.6.2. Nasal clearance study

New Zealand white rabbits weighing 3±0.3 kg were used. Sedation occurred with Rompun® (0.2 ml/kg) and the rabbits were anesthetized with ketamine (15 mg/kg). Anesthesia was necessary to immobilize the rabbits during recording the dynamic images. Ten milligram DDWM/Carbopol® 974 P (9/1) containing 1–2 MBq sodium pertechnetate was administered to the right nostril of three rabbits using the delivery system described in Section 2.4. The deposition and subsequent clearance of the powder formulation was followed by gamma scintigraphy using a HELIX camera (Marconi Medical Systems, Cleveland, OH, USA). Dynamic lateral views of the rabbits were recorded at appropriate time intervals during 150 min post administration. The images were recorded for subsequent analysis and quantification. Circular regions of interest (ROI) were defined around the nasal cavity and the whole body of the rabbits for quantification of the data. The count rate from each ROI, corrected for radioactive decay and background, was then expressed as a proportion of the highest count rate, obtained from the image recorded in the nasal cavity ROI immediately after dosing. The clearance of the formulation was evaluated as a decrease in percentage activity against time for each rabbit. The study had approval from the Ethical Committee of Laboratory Animals (Ghent University, Belgium).

3. Results and discussion

Research has already been done on the bioavailability enhancement of peptides through the nasal route (Dondeti et al., 1995; Chandler et al., 1994; Fernández-Urrusuno et al., 1999). In these studies absorption enhancers, enzyme inhibitors and bioadhesives were evaluated after a single administration of the formulation, while no data are available on the possible changes in peptide bioavailability after multiple nasal administrations.

In the first experiment insulin was administered nasally to rabbits on the first day using the co-spray dried mixture of Amioca® starch and Carbopol® 974P (1/3) and an absolute bioavailability of 17.8±4.5% was obtained (Table 2; Fig. 1). The blood glucose levels decreased by 55.7±6.3% after 90 min while 180 min after the insulin administration the blood glucose value remained 51.1±13.3% of the initial value (Fig. 2). When insulin was administered on the first day using the mixture of drum dried waxy maize starch and Carbopol® 974P (9/1) an absolute bioavailability of 13.4±3.2% was calculated (Fig. 1). The blood glucose levels dropped by 49.9±11.5% after 78 min, while 180 min after the administration, the blood glucose recovered 86.6±19.2% of the initial value (Fig. 2). From the second till the seventh day, the powder formulation (containing no insulin) was daily administered to the rabbits and no blood samples were taken. On day 8, insulin was again administered using the same formulations. With the co-spray dried mixture of Amioca® starch and Carbopol® 974P (1/3) an absolute bioavailability of only 4.4±1.1% was obtained (Fig. 1) and being significantly ($P < 0.001$) lower compared with the first day. Also the decrease of the blood glucose levels was not as pronounced after 8 days, with a maximal decrease of 33.1±7.9% after 90 min (Fig. 2). Besides there was a sharper rise of the blood glucose levels after 180 min on the eighth day compared with the first day of administration, with a mean blood glucose value of 95.3±18.1% of the initial value. The lower insulin bioavailability and the lower decrease of the blood glucose levels after multiple administrations were also observed with the formulation consisting of drum dried waxy maize starch and Carbopol® 974P (9/1). The absolute bioavailability (3.6±0.6%) was significantly decreased ($P < 0.001$) compared with the first day of the experiment (Fig. 1). The maximal blood glucose level was also less decreased (30.5±

Table 2
Absolute bioavailability and maximal blood glucose decrease after administration of 8.7 IU insulin per nostril using a co-spray dried mixture of Amioca® starch and Carbopol® 974P (1/3) and a physical mixture of drum dried waxy maize starch and Carbopol® 974P (9/1) as the bioadhesive carrier

	Amioca/Carb. 1/3		DDWM/Carb. 9/1	
	Day 1	Day 8	Day 1	Day 8
Experiment 1				
Absolute bioavailability (%)	17.8±4.5	4.4±1.1***	13.4±3.2	3.6±0.6***
Max blood glucose decrease (%)	55.7±6.3	33.1±7.2	49.9±11.5	30.5±11.1
Experiment 2				
Absolute bioavailability (%)	10.2±2.8	11.9±2.8	–	–
Max blood glucose decrease (%)	60.3±20.7	55.0±19.6	–	–
Experiment 3				
Absolute bioavailability (%)	–	4.0±1.2***	–	–
Max blood glucose decrease (%)	–	36.5±10.6	–	–

****, Significantly different in comparison of the bioavailability obtained on day 1, with $P \leq 0.001$. Data are presented as the mean (±S.D., $n = 6–8$).

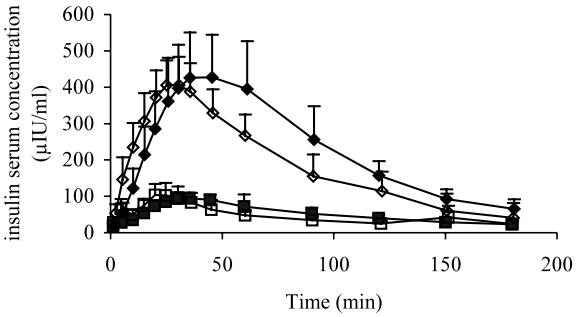

Fig. 1. Insulin serum concentration–time profile in rabbits after 1 (diamonds) and 8 (squares) days of nasal administration of a bioadhesive powders consisting of the co-spray dried mixture of Amioca® starch and Carbopol® 974P (1/3) (filled symbols) and drum dried waxy maize starch and Carbopol® 974P (9/1) (open symbols) (experiment 1). Data are presented as the mean (±S.D., $n = 8$).

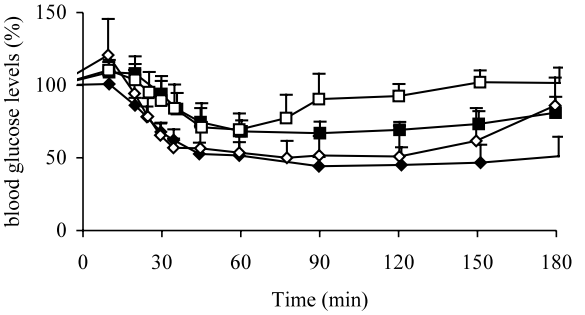

Fig. 2. Decrease of the blood glucose levels, expressed as a percentage of the initial value, after 1 (diamonds) and 8 (squares) days of nasal administration of the bioadhesive powder consisting of the co-spray dried mixture of Amioca® starch and Carbopol® 974P (1/3) (filled symbols) and drum dried waxy maize starch and Carbopol® 974P (9/1) (open symbols) (experiment 1). Data are presented as the mean (±S.D., $n = 8$).

11.1% after 60 min) compared with the first day. After 180 min the blood glucose levels reached 101.4±10.6% of their initial value (Fig. 2).

The high local viscosity after dispersion of the powder formulations in the mucus, slows down the mucociliary clearance (Cornaz and Buri, 1994; Edman and Björk, 1992). Initially this decrease in mucociliary clearance results in a longer residence time of the formulations in the nasal cavity and consequently in a longer available absorption time for the drug (Illum et al., 1994). The scintigraphic study on rabbits revealed that 81.9±12.7% of the powder formulation DDWM/Carbopol® 974P 9/1 was still detected in the nasal cavity 150 min after a single administration and indicated that indeed the mucociliary clearance was decreased when this formulation was administered in the nasal cavity as the normal mucociliary clearance is about 10 min in rabbits (Gizurarson, 1993b).

The viscosity of an 8% mucin dispersion in simulated nasal fluid was 2.1E-03±8.7E-05 Pa when an oscillation stress of 1.4 Pa was applied. An increase of the G'' value of 281±91 Pa and 17.6±2.5 Pa was obtained when a dispersion of mucin and the co-spray dried mixture of Amioca® starch and Carbopol® 974P (1/3) or the mixture of drum dried waxy maize starch and Carbopol® 974P (9/1) was tested, respectively. This dramatic increase of viscosity resulted for both powders in a sufficient increase of the nasal residence time so that insulin uptake could occur but could result in an incomplete clearance of the bioadhesive powder after 24 h and so progressively decelerating the mucociliary clearance. This might result in a progressive accumulation of material forming a barrier against drug absorption. When the nasal cavity was examined 24 h after the last powder administration, stickiness of the turbinates was observed and part of the bioadhesive powder formulation remained in the nasal cavity. The initial advantage of a longer residence time of the powder formulation might turn into a disadvantage after multiple administration and impart bioavailability. Long residence times of the powder formulations were also reported by Ugwoke et al. (2000a,b) who noticed nasal residence times of more than 24 h using powder formulations containing Carbopol® 971 P and carboxymethylcellulose.

The promising bioavailability values obtained on the first day could also be due to an opening of the tight junctions procured by the administration of powder formulations (Edman et al., 1992). On the other hand the accumulation of powder in the nasal cavity might prevent the opening of the tight junctions during further administrations.

To support the theory of incomplete clearance of the powder a second experiment was performed with the co-spray dried mixture of Amioca® starch and Carbopol® 974P (1/3). In this experiment insulin containing powder was administered on the first and the eighth day of the experiment, while no powder was administered in the intervening days. A bioavailability of 10.2±2.8% was obtained for the nasally administered insulin with a maximal decrease of the blood glucose level of 60.3±20.6% after 120 min while 180 min after the insulin administration the blood glucose level was still 48.3±19.5% of the initial value. Eight days after this first insulin administration, the bioavailability of the second insulin administration was 11.9±2.8% (Table 2; Fig. 3). The blood glucose decreased with 55.0±19.6% after 90 min but recovered after 180 min till 56.2±17.8% of its initial value (Table 2; Fig. 4). The bioavailability after 8 days was not significantly different compared with the bioavailability obtained on the first day of administration of experiment 2 and to the bioavailability of the first day of experiment 1 ($P > 0.05$). The lower mean value of the bioavailability in experiment 1 compared with experiment 2 could only be attributed to differences between groups.

As human insulin was used in our experiment, the lower bioavailability after multiple administration could have been due to the generation of antibodies in the rabbits against the human insulin. Therefore, in a third experiment, the powder formulation consisting of the co-spray dried mixture of Amioca® starch and Carbopol® 974P (1/3) (without insulin) was administered daily from the first day of the experiment.

Administration of the powder formulation with insulin on day 8 only resulted in a bioavailability of 4.0±1.2% (Table 2). The blood glucose dropped to 63.5±10.6% of the initial value after 60 min and increased to 74.8±19.6% of its initial value after 180 min. This lower bioavailability was

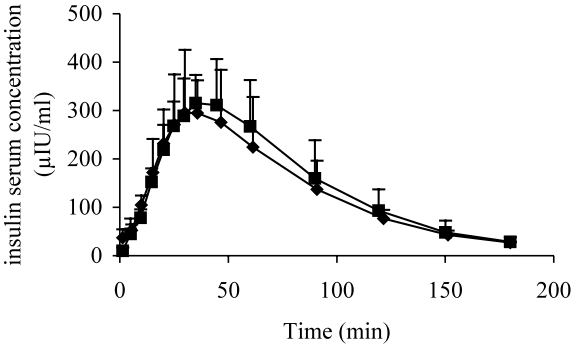

Fig. 3. Insulin serum concentration–time profile in rabbits after 1 (◆) and 8 (■) days of nasal administration of the bioadhesive powder consisting of the co-spray dried mixture of Amioca® starch and Carbopol® 974P (1/3) (experiment 2). Data are presented as the mean (±S.D., $n = 6$).

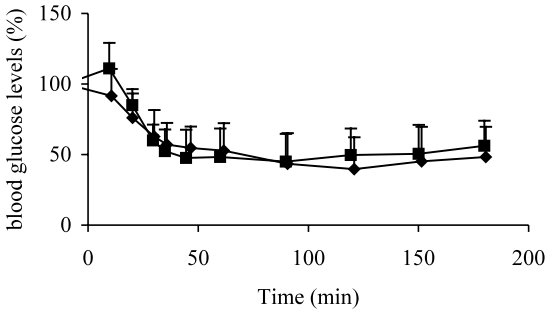

Fig. 4. Decrease of the blood glucose levels, expressed as a percentage of the initial value, after 1 (◆) and 8 (■) days of nasal administration of the bioadhesive powder consisting of the co-spray dried mixture of Amioca® starch and Carbopol® 974P (1/3) (experiment 2). Data are presented as the mean (± S.D., $n = 6$).

obviously not due to antibody formation against insulin, as it was administered only once, but must probably due to the accumulation of the powder formulation because of an incomplete clearance of the nasally administered powder. The influence of irritation after a chronic administration of the powder formulations can be ruled out as no change of the epithelium barrier was observed even after a 4 weeks administration of the powder formulation in rabbits (Callens et al., 2001).

4. Conclusions

A nasal bioavailability of insulin above 10% was obtained after a single administration of the bioadhesive platforms composed of the co-spray dried mixture of Amioca® starch and Carbopol® 974P (1/3) or a physical mixture of drum dried waxy maize starch and Carbopol® 974P (9/1). Multiple administration of the bioadhesive powders caused a dramatic reduction of the nasal insulin bioavailability and a lower decrease of the blood glucose levels in rabbits. This lower bioavailability was mainly due to the high viscosity of the bioadhesive powders in the nasal mucus, causing a physical barrier towards absorption and a strongly decelerated mucociliary clearance.

Acknowledgements

The authors are very grateful to D. Tensy for his excellent assistance in the rabbit experiments. C. Callens acknowledges her position of Research Assistant of the IWT, Belgium.

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