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Degradable starch microspheres as a nasal delivery system for insulin

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Summary

Degradable starch microspheres (DSM) with a diameter of 45 μm were investigated as a nasal delivery system for insulin in rats. Insulin (0.75 IU/kg and 1.70 IU/kg)–DSM preparations administered nasally as a dry powder resulted in a dose-dependent decrease in blood glucose and a concomitant increase in serum insulin. The blood glucose was reduced within 30–40 min by 40% and 64%, respectively, using the aforementioned doses. The glucose level was normalized after 4 h. The insulin peak was reached 8 min after dosing. The bioavailability was approx. 30%. DSM alone or soluble insulin had no effect on these parameters. The results obtained indicate that DSM offer a system for improving the nasal absorption of drugs.

Introduction

Nasal administration of drugs is not a new concept. Many drugs, in particular hormones, have been given nasally for many years (Chien, 1985). However, a renewed interest in this route has been apparent during the past decade. New potent peptides and macromolecular drugs are generally administered parenterally. The nasal route offers an attractive alternative in these cases.

The histology of the nasal mucosa reveals that the permeability for molecules, even macromolecules is several times higher compared with the intestinal mucosa. Furthermore, a well-developed vascular bed and a large surface area will favour the permeation of nasally administered drugs (McMartin et al., 1987). The nasal route (i.n.) is also beneficial for drugs with a high hepatic first-pass metabolism. Further, the nasal route lends itself well for self-medication. Many drugs have been tested by this route, e.g. insulin (Hirai et al., 1978; Hirata et al., 1978), oxytocin (Hendricks et al., 1960), LHRH (Solbach et al., 1973), Desmopressin (Harris et al., 1986), steroids (Hussain et al., 1984), and also low-molecular drugs (Hussain et al., 1979; Duchatean et al., 1986a). It has also been shown that a quaternary ammonium compound (Su et al., 1984) is completely absorbed by the nasal mucosa. The greatest disadvantage with the nasal route has been the low bioavailability for macromolecules (Fischer et al., 1987). Attempts have thus been made to increase the uptake, e.g. by addition of different absorption-promoting substances like bile salts (Moses et al., 1983;

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Duchetean et al., 1986b), and surfactants (Hirai et al., 1981a). Other enhancer systems like polyacrylic gels have also been tried (Morimoto et al., 1985). However, the toxicity of these substances to the nasal mucosa precludes long-term treatment.

Recently, a new concept was introduced by Illum et al. (1987). They proposed the use of degradable starch microspheres (DSM) with a diameter of 45 μm as a nasal delivery system for drugs.

The present study was undertaken to investigate the potential value of DSM as a nasal carrier system for insulin in rat. Insulin is used as model substance because it has been widely used to evaluate different nasal drug delivery systems.

Materials and Methods

Preparation of microspheres

Human monocomponent insulin from Novo (Denmark) was used. 80 μl insulin (100 IU/ml) was mixed with 100 mg degradable starch microspheres (DSM), from Pharmacia (Sweden). The gel was freeze-dried overnight and then passed through a 63 μm sieve to give a homogeneous powder. Insulin activity (0.25 IU/mg) was determined and the DSM–insulin powder was stored in a dry place at 8° C until use. The freeze-drying process did not change the shape, size or other parameters of the DSM.

Animal experiments

Male Sprague–Dawley (Alab AB Sweden) rats weighing 200–250 g were used throughout. The rats were fasted for 15–17 h prior to the experiments. They were anesthetized with an intraperitoneal (i.p.) injection of thiobutabarbital-sodium (120 mg/kg) (Inaktin BYK), and placed on hot-plates to maintain a body temperature of 37° C. The trachea was cannulated and arteria carotis was catheterized with a polyethylene tube. The experiment was started 30 min after operation. The intravenous (i.v.) injections were made through a catheter in the external vena jugularis. The solutions and powders were administered through the nostril with a polyethylene tube, PE-90. The test substance was put in the tube and weighed. It was introduced by blowing air from a syringe. Blood samples of 50 μl for blood-glucose or 200 μl for insulin determinations were withdrawn from the arteria carotis. Serum was isolated by centrifugation at 3000 rpm and supplemented with 10 μl aprotinin (10,000 KIE/ml) and stored at −20° C until insulin analysis. 2 × 5 groups with 5–8 rats in each were used: (1) soluble insulin 2.0 IU/kg i.n.; (2) DSM 0.5 mg/kg i.n.; (3) DSM–insulin 0.75 IU/kg i.n.; (4) DSM–insulin 1.70 IU/kg i.n.; and (5) soluble insulin 0.25 and 1.0 IU/kg i.v.

Analysis

Blood glucose level was measured by a glucose-oxidase method (GLOX from Kabi) and serum insulin with a radioimmunoassay (RIA) (Pharmacia insulin RIA 100) (Livesey et al., 1980).

Calculations

The areas under curves (AUC) were calculated with the trapezoidal rule extrapolating to infinity the last C/k_e. Blood glucose and serum insulin values from rats receiving empty DSM were used as controls when calculating the parameters. Statistical significance was calculated according to One-way ANOVA following the Student–Newman–Keuls test.

Results

Nasal administration of soluble insulin (2 IU/kg) to rats had no effect on the blood glucose and serum insulin levels (Figs. 1 and 2). Insulin administered in DSM as a dry powder (0.75 IU and 1.7 IU/kg) by the same route resulted in a dose-dependent decrease in blood glucose (Fig. 1). The maximal decrease is reached approx. 40 min after dosing, irrespective of the dose given. The reduction in the blood glucose level after doses of 0.75 IU/kg and 1.7 IU/kg was 40–64%, respectively (Table 1). The glucose level normalized after 4 h. DSM alone had no effect on the blood glucose level. The decrease in blood glucose level when using insulin in DSM was significantly different ($P < 0.01$) from that for soluble insulin and DSM alone during the intervals 20–120 min

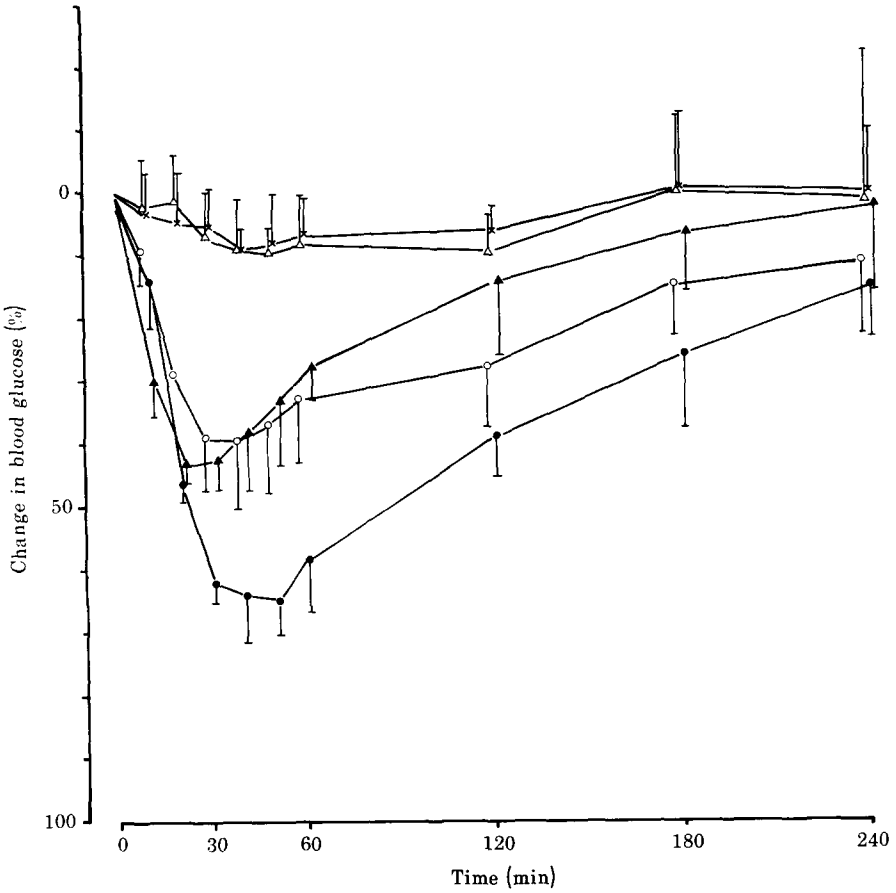

Fig. 1. Change in blood glucose in rats after intravenous or intranasal administration of different insulin preparations. Soluble insulin 2.0 IU/kg i.n. (△); soluble insulin 0.25 IU/kg i.v. (▲); DSM-insulin 0.75 IU/kg i.n. (○); DSM-insulin 1.70 IU/kg i.n. (●); empty DSM 0.5 mg/kg i.n. (×). The data are expressed as mean ± S.D.; 6–8 animals were used in each group.

(DSM-insulin 0.7 IU/kg) and 20–180 min (DSM-insulin 1.7 IU/kg). A significant difference was seen between the doses during the interval 20–60 min.

Intravenous administration of insulin (1 IU/kg) resulted in a rapid reduction of blood glucose. The maximal effect was seen after 20 min. The level was normalized more rapidly than with insulin in DSM i.n.

To calculate the bioavailability of insulin given

TABLE 1

Parameters for 2 doses of DSM-insulin given i.n.

BA = bioavailability. All figures are expressed as mean ± S.D. ($n = 6–8$).

Dose (IU/kg)	Serum insulin				Blood glucose	
	AUC (μU · h/ml)	Peak level (μU/ml)	Peak time (min)	*BA* (%)	Maximal decrease (%)	Peak time (min)
0.75	66 ± 23	146 ± 64	8.0 ± 1.5	30	40 ± 13	36 ± 12
1.70	165 ± 97	167 ± 46	7.3 ± 2.6	33	64 ± 8	38 ± 8

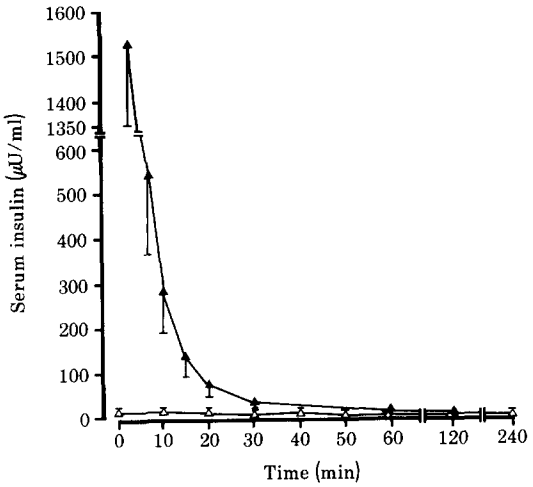

Fig. 2. Change in serum insulin in rats after intranasal and intravenous administration of soluble insulin, 2.0 IU/kg i.n. (△); 1.0 IU/kg i.v. (▲). The data are expressed as mean ± S.D. ($n = 6$).

nasally with DSM, insulin was injected i.v. at a dose of 0.25 IU/kg, (Fig. 2). The insulin level decreases rapidly and a normal value is reached after 30–60 min. The serum insulin levels for insulin administered with DSM is shown in Fig. 3. The peak values are reached at approx. 8 min after dosing, irrespective of dose. The peak value and *AUC* are dose dependent (see Table 1). The absolute bioavailability was calculated to be 30% and 33% for the two doses used. With the doses used, the serum insulin concentrations differ significantly from the controls ($P < 0.01$) (soluble insulin and empty DSM) 3–20 min and 3–30 min post-dosing for the low and the high doses, respectively.

Discussion

The results of this study show that starch microspheres with a mean diameter of 45 μm increase the nasal absorption of co-administered insulin in rats. A clear dose–response relationship is obtained when changing the insulin dose showing a systemic absorption of insulin in a pharma-

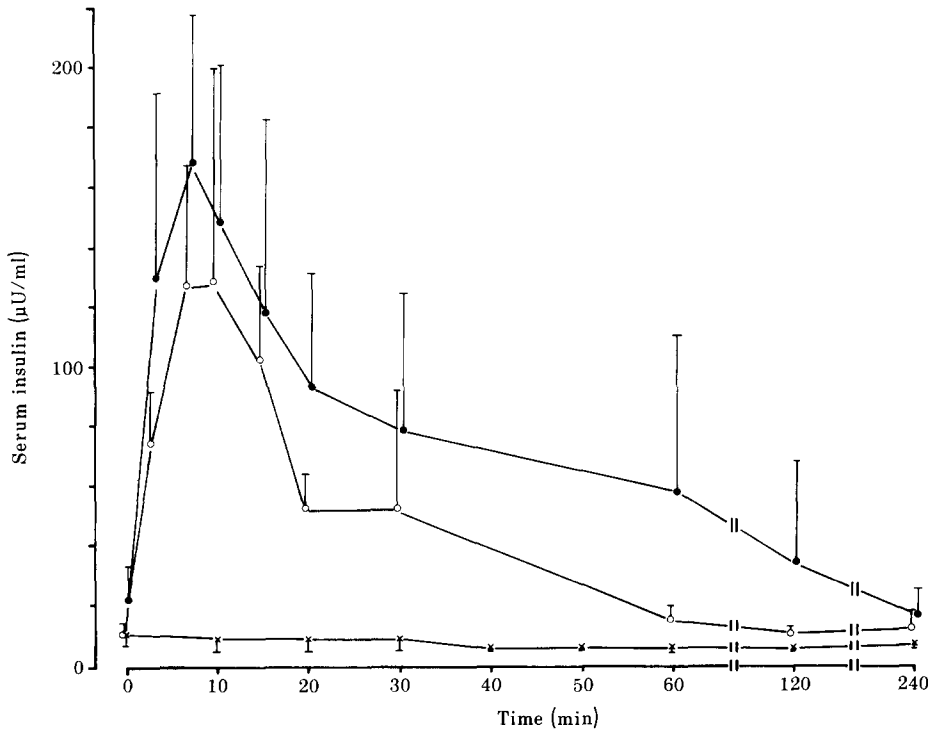

Fig. 3. Change in serum insulin in rats after nasal administration of: DSM–insulin 0.75 IU/kg (○); DSM-insulin 1.7 IU/kg (●); empty DSM 0.5 mg/kg (×). The data are expressed as mean ± S.D. ($n = 6$).

cologically active form. The peak serum concentration of insulin is obtained 7–10 min after dosing, indicating a rapid release of insulin from the spheres with a concomitantly rapid transport across the nasal mucosa. The maximal decrease in blood glucose level is reached 30–40 min after administration of the insulin microspheres. The effect on the blood glucose is delayed by approx. 30 min relative to the insulin peak. Similar results have been reported earlier both in humans and animals when insulin was administered nasally by aerosols or by another experimental system (Moses et al., 1983). The absolute bioavailability obtained with the microsphere system was approx. 30%, showing that the starch microspheres (DSM) strongly promote the absorption.

This promoting effect mediated by the DSM (starch microspheres) is comparable with those effects obtained with surfactants, different gel systems and powder dosage forms (Hirai et al., 1981a; Morimoto et al., 1985; Nagai et al., 1984). However, it should be emphasized that the hypoglycemic effect seen in this study with microspheres is achieved at a dose range (0.75–1.70 IU/kg) which is considerably lower than reported by Hirai et al. (1981a) who used 10 IU/kg to obtain a similar reduction in blood glucose level.

Illum et al. (1987) have recently shown, in a γ-scintigraphy study in man that starch microspheres, given i.n. as a dry powder, are eliminated slowly from the nasal cavity. The reason is probably that the spheres swell in contact with the nasal mucosa resulting in gels. The exact mechanism is obscure but a plausible explanation is that the spheres do not swell completely owing to the limited amount of moisture in the nasal cavity. Since these "gels" are not fully swelled, they are eliminated only by the mucocilliary movements and not by drainage. Another factor which can contribute to the low clearance is the muco-adhesiveness of the spheres.

The fact that the spheres are not fully swelled will also affect the release of insulin from the spheres. If an excess of water is available, the release of insulin is instantaneous. The slow clearance of the spheres and the limited humidity in the nose result in an increased contact time between insulin and the mucosa.

Several muco-adhesive systems have been proposed and used intranasally, e.g. polyacrylic gel (Morimoto et al., 1985), cellulose derivatives and neutralized Carbopool 934 (Nagai et al., 1984). Experiments have also been performed in humans with bile salts (Moses et al., 1983; Hirata et al., 1978; Longenecker et al., 1987). A rapid increase in insulin concentration and a decrease in blood glucose were seen in both patients and healthy volunteers. However, the use of bile salts as a nasal enhancer is uncertain because of the alterations seen in the microvilli after prolonged treatment with sodium glycocholate (Hirata et al., 1978; Hirai et al., 1981b).

The rat model described by Hirai et al. (1981c) has been widely used to study nasal administration of drugs. However, with this technique, several steps are taken to prevent drainage of drug solution from the nasal cavity, e.g. blocking of the nostril and the nasopalatine with an adhesive glue. In this study, care was taken to maintain the normal function of the cavity by minimizing disturbances of the mucosa via mechanical manipulation. The dose was introduced into the nostril through a small polyethylene tube (PE-90) by means of a gentle puff of air.

It is apparent from this study that DSM (starch microspheres) offer an efficient delivery system for insulin in rats. The rationale for this effect is unclear but probably the bioadhesive character and the swelling properties of the spheres are important factors (Illum et al., 1987).

To elucidate the potential of starch microspheres as a nasal delivery system for drugs, the following studies are proposed: (1) morphological studies on the nasal mucosa after treatment with microspheres at different doses for longer times; (2) the optimal ratio between amount of drug and amount of spheres; (3) immunological aspects especially when peptides and therapeutic proteins are to be given together with microspheres; and (4) check whether the spheres are degraded by enzymes in mucosa layer or whether they are cleared intact for the nose. Probably several other factors have also to be considered but these proposed activities are essential.

The present results indicate that starch microspheres may have interesting applications as a

nasal delivery system for low molecular drugs, peptides, or small proteins.

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