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Characterization of degradable starch microspheres as a nasal delivery system for drugs

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Summary

Intranasal administration of starch microspheres and insulin as a dry powder results in a rapid decrease in plasma glucose. The optimal dose of spheres for a given dose of insulin is approx. 3–7 mg per kg body weight in the rat. The degree of cross-linking governs the water uptake and swelling and thereby the release of insulin from the spheres in vitro. No significant differences could be seen in vivo between spheres with different swelling factors. Starch microspheres do not induce erythrocyte hemolysis. A comparison between microspheres and starch powders (molecular weights: 25 000 and 11 000, respectively) shows that the insoluble starch of mol. wt. 25 000 and the microspheres reduce the plasma glucose level to the same extent. Water-soluble starch powder (mol. wt. 11 000) does not affect the plasma glucose level. The crucial properties for the absorption-promoting effect of microspheres are water absorption and water insolubility.

Introduction

Macromolecular drugs, e.g. peptides and proteins, are commonly administered parenterally. There are several drawbacks to this route of administration. Firstly, injections must be given by trained staff, secondly there is a risk of infection and thirdly patients may express a phobia for needles and syringes. Extensive pharmaceutical research has been devoted to finding alternative routes, and intranasal administration has attracted much interest. However, as the mechanism of transport of drugs through the nasal mucosa is not fully understood, and since transport is largely dependent on the molecular size (McMartin et al., 1987), the bioavailability is low. Therefore, there is a need for absorption enhancers. The most commonly used enhancers such as bile acids and surfactants are all associated with toxic effects on the nasal mucosa. An interesting system based on swellable microspheres has been introduced by Illum et al. (1987). When insulin was administered with the system in rats, a bioavailability of 30% was achieved (Björk and Edman, 1988).

We have now studied the optimal ratio between spheres and insulin, compared spheres with different properties and tested the biocompatibility of the spheres. The microspheres have also been compared with other powder formulations.

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Materials and Methods

Materials

Sodium taurodeoxycholate and sodium taurocholate were purchased from Sigma, U.S.A. All other chemicals were of analytical grade.

Preparation of microspheres and powders

Degradable epichlorohydrin cross-linked starch microspheres (DSM) with a mean diameter of 45 μm (Pharmacia AB) and starch of molecular weight 25 000 and 11 000 (Reppe Glykos AB, Sweden) were mixed with human monocomponent insulin (100 IU/ml) Novo, Denmark) in the proportions 100 mg/80 ml. The gel was freeze-dried and passed through a 63 μm sieve to yield a homogeneous powder. Insulin activity was determined by radioimmunoassay (RIA) (Pharmacia Insulin RIA 100) (Livesey et al., 1980).

By adding 'empty' microspheres to insulin-loaded microspheres preparations were obtained from 0.43 IU/mg to 0.10 IU/mg.

The same procedure was used for the other two DSMs. All DSMs were of similar size, but due to differing degrees of cross-linking, they had different swelling factors. Spheres of low swelling degree are highly cross-linked, whereas those of high swelling degree are loosely cross-linked. The swelling factor is defined as the bead volume in cm^3 obtained when 1 g DSM is allowed to swell in buffer. The DSM used earlier had a swelling factor of 8–10 and the other two had factors of 5 and 17, respectively. They are referred to as DSM 5 or DSM 17. Starches of molecular weight 11 000 or 25 000 were prepared in the same way. The latter starch was used for synthesising the DSMs.

In vitro release

The kinetics of release of insulin from DSM of different degrees of cross-linking and therefore having different swelling factors were measured. 10 mg of the DSM-insulin preparations were placed in a diffusion chamber (Fig. 1). The chamber was immersed in a water bath (37 °C) and samples of 100 μl were withdrawn after 3, 5, 10, 15, 30, 60 and 120 min. After each sampling, the volume was replaced with physiological saline.

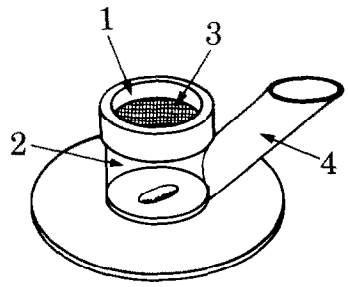

Fig. 1. Diffusion apparatus for determining water uptake and release of insulin from starch spheres. (1) Donor compartment, (2) receiving compartment, (3) membrane, (4) sampling site.

The protein content was determined with the Folin Lowry assay (Lowry et al., 1951).

Erythrocyte hemolysis

The experiment was conducted essentially as described by Hirai et al. (1981b). 10 ml of fresh rabbit blood was centrifuged at 1000 × *g* for 10 min. The cells were then washed three times by suspending them in 5 ml of 0.9% NaCl and centrifugation (800 × *g*, 5 min). They were then suspended in 50 ml of 0.9% NaCl. 0.2 ml cell suspension was incubated with 5 ml of each sample for 5 min at room temperature. The test tubes were placed in ice and then centrifuged at 800 × *g* for 5 min. The absorbance of the supernatant was recorded at 540 nm. 100% hemolysis was measured by adding 5 ml deionized water to 0.2 ml cell suspension. The percentage of total hemolysis was then calculated.

Animal experiments

Experiments were performed as described previously (Björk and Edman, 1988). Male Sprague-Dawley rats (Alab AB, Sweden), weighing 200–250 g each, were used. They were fasted 15–17 h before operation to standardize the plasma glucose level. Inaktin^R Byk Gulden (thiobutabarbital sodium) was given i.p. at a dose of 120 mg/kg. Immediately after sedation, the rats were laid on their backs on heated plates to maintain a body temperature of 37 °C throughout the experiment. Trachea and arteria carotis were cannulated with PE 200 and PE 50 polyethylene tubing, respectively. Administration started 30 min post-operation. The test substances (microspheres and starch

powders) were placed in a pre-weighed, small polyethylene tube (PE 90). The tubing was carefully inserted through the nostrils and into the nasal cavity. The dose was administered by blowing air from a syringe through the tube.

Blood samples (100 μl) were withdrawn from the arteria carotis at intervals during 4 h. After centrifugation at 3000 rpm, the plasma was withdrawn and frozen until glucose analysis.

Analysis

Plasma glucose levels were measured using hexokinase and glucose-6-phosphate dehydrogenase (Beckman Dri-STAT).

Calculations

The areas under curves (AUC) were calculated with trapezoidal rule. Statistical significance was calculated according to one-way ANOVA following the Student-Newman-Keuls test. The decrease in plasma glucose level (D, total decrease) was calculated according to Hirai et al. (1981a).

$$D\% = \frac{AUC_c - AUC_I}{AUC_c} \times 100$$

AUC_c and AUC_I = area under plasma glucose vs time curve from 0 to 4 h after nasal administration of DSM and DSM + insulin, respectively.

Results

Administration of degradable microspheres together with insulin intranasally significantly reduced the blood glucose level. To establish the ratio between a given dose of insulin and microspheres giving maximal bioavailability, one unit of insulin was mixed with different amounts of DSM and administered at doses of one unit insulin/2.35–10 mg spheres per kg body weight.

In Fig. 2, the optimal ratio between insulin and spheres was observed at around 3–7 mg spheres per kg body weight. Lower or higher doses of microspheres reduced the effect of insulin on the plasma glucose level.

Degradable starch microspheres (DSM) crosslinked with epichlorhydrin have a swelling factor

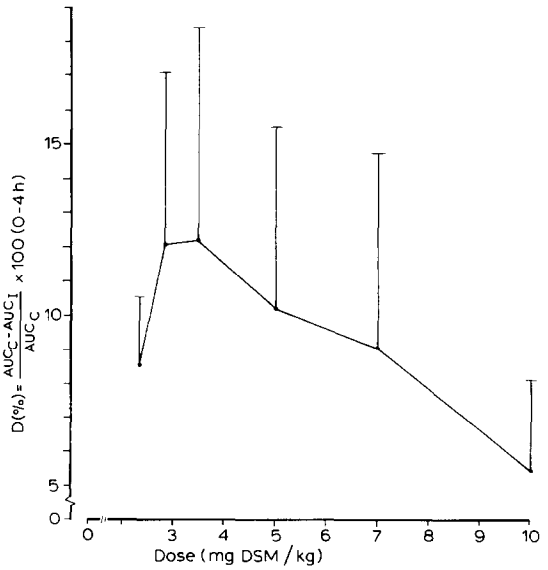

Fig. 2. Effect of different doses of starch microspheres (mg/kg body weight) on the plasma glucose level when administered together with a given amount of insulin (1 IU/kg body weight). The effect was calculated by comparing AUC values from empty microspheres (10 mg/kg body weight (AUC_c)), and the combination microspheres (2.85–10 mg/kg) and insulin (AUC_I). The AUC are defined as the area under plasma glucose vs time curve for 4 h. Each point represents data from 6 animals. The vertical bars show standard deviations of the mean.

of 8–10. To investigate the importance of this factor and determine how it influences the pharmacodynamic effect of insulin, starch microspheres with swelling factors of 5 and 17 were also tested, both in vitro and in vivo. The kinetics of insulin release are shown in Fig. 3a. Spheres with a swelling factor of 5 release 90% of the insulin within 10 min, whereas those with a swelling factor of 17 release a comparable amount of insulin after 50–60 min. No significant difference could be seen between spheres with factors 5 or 8.

Fig. 3b shows the in vivo effects in rats of spheres with different swelling factors. All three preparations lowered the plasma glucose level. The maximal decrease was obtained approx. 30–40 min after administration irrespective of the type of sphere used. No significant difference could be seen between the different spheres. The insulin dose given was 1 IU per kg body weight and the dose of spheres was 3.5 mg per kg body weight.

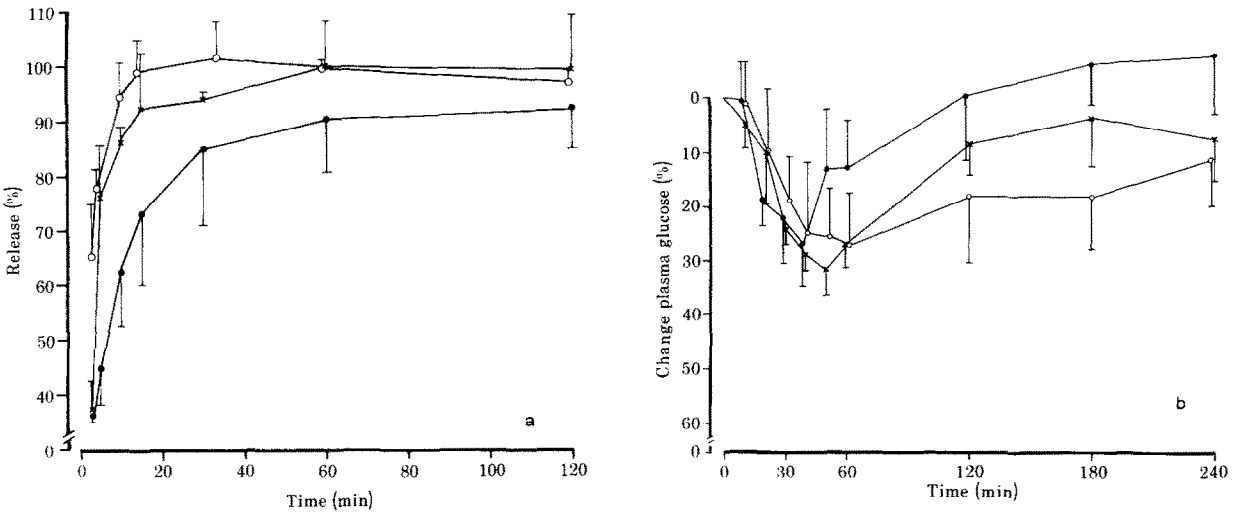

Fig. 3. (a) Release of insulin in vitro from degradable starch microspheres (DSM) with different swelling factors: (X) DSM 8, (○) DSM 5 and (●) DSM 17. Each point represents three experiments. The vertical bars show standard deviations of the mean. (b) Change in plasma glucose after nasal administration of degradable starch microspheres (DSM) with different swelling factors and insulin (1 IU/kg body weight). The spheres used were: (X) DSM 8; (○) DSM 5; and (●) DSM 17. Each point represents a mean of 3 determinations. The vertical bars show standard deviations of the mean.

A comparison was also made between DSM and two types of starch. The starches used had molecular weights of 25 000 and 11 000, respectively. Starch with a mol. wt. of 25 000 is insoluble in water whereas that of mol. wt. 11 000 is soluble. Starch of mol. wt. 25 000 was used to prepare DSM. The insulin dose was 1 IU per kg body weight and the dose of starch or spheres was 4 mg/kg body weight. Soluble starch (mol. wt. 11 000) had no effect on the blood glucose level (Fig. 4). Insulin administered in combination with DSM or with insoluble starch (mol. wt. 25 000) as a dry powder resulted in a rapid decrease in plasma glucose. The reduction in plasma glucose was approx. 45–50% and occurred 30–40 min after administration. Normal glucose levels were reached 4 h after dosing. No significant difference could be seen between starch of mol. wt. 25 000 and DSM.

DSM did not induce erythrocyte hemolysis under the conditions used. All DSM preparations gave a pH of 7.2–7.4 when swelled in distilled water. Control agents such as sodium taurocholate and sodium taurodeoxycholate gave 50% hemoly-

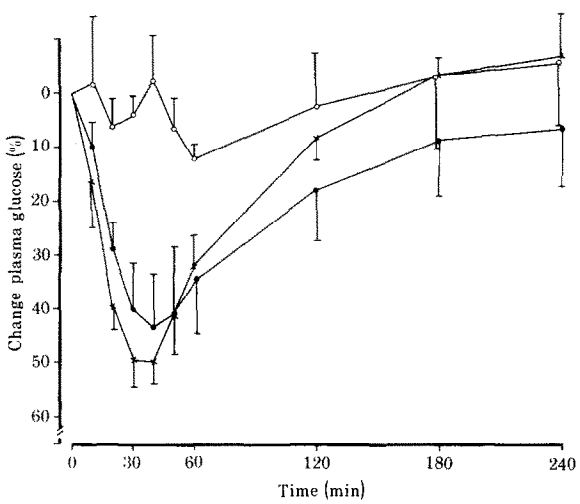

Fig. 4. Change in plasma glucose after nasal administration of degradable starch microspheres (DSM) and starch powders together with insulin. The insulin dose was 1 IU per kg body weight. The amount of spheres and starch was 4 mg per kg body weight. The following preparations were used: (X) degradable starch microspheres, DSM; (○) water-soluble starch of mol. wt. 11 000 and (●) water-insoluble starch of mol. wt. 25 000. The points represent the mean and standard deviation of the mean from six animals.

sis at concentrations of 0.6 and 9.1 mg/ml, respectively.

Discussion

The concept of intranasal administration of degradable starch microspheres was introduced by Illum et al. (1987). The spheres promote the absorption of both macromolecular and low molecular weight drugs (Edman and Björk, 1988; Illum et al., 1988) but the exact mechanism is not clear. However, the swelling of the spheres is critical for the enhancement of drug transport across nasal mucosa. The optimal amount of microspheres for a given dose of insulin was found to be 3–7 mg/kg body weight in rats. Lower or higher doses of microspheres decreased the promoting effect. The swelling of the microspheres depends on the amount of water in the nose and the amount of spheres given. Excess water or a small amount of spheres in relation to the humidity in the nose will result in an instantaneous release of insulin. Since the spheres do not act as a slow release depot in a swelled state, no effect on the plasma glucose level will be seen. If the amount of microspheres exceeds the moisture available for swelling, then a proportion will remain dry and the insulin will not be released. The optimal conditions are presumably such that the microspheres are all just fully swollen.

The water uptake is another parameter which can influence the efficacy of this delivery system. In vitro experiments in a diffusion chamber show that the insulin release depends on the degree of cross-linking and thereby the swelling factor. In this study, starch microspheres with different swelling factors (5, 8 or 17) released insulin at different rates. Microspheres with a high swelling factor release insulin slowest while those with low swelling factors release more rapidly.

The reason is probably that more water is needed for spheres with a low degree of cross-linking before the insulin incorporated in the sphere dissolves and diffuses out from the sphere matrix. However, even if a difference in rates does exist in vitro, no significant difference could be seen in the animal model (Fig. 3b). Presumably the rate of water uptake in vivo is not critical, at least not within the interval 10–60 min.

To characterize further this microsphere system, comparisons were made with particles of water-insoluble starch (mol. wt. 25 000) and water-soluble starch (mol. wt. 11 000). Fig. 4 reveals that starch particles of mol. wt. 25 000 give the same decrease in plasma glucose as that with the microsphere system. However, water-soluble starch with an equivalent dose of insulin had no effect on plasma glucose levels. These findings are noteworthy as they show that the system must be water-absorptive and water-insoluble.

Similar decreases in plasma glucose were observed with both water-insoluble starch and DSM. Similar findings were reported by Nagai et al. (1984). Both systems absorb water but the former system is less well characterized. It is easier to administer microspheres in a reproducible way than starch powder. Further, DSMs have a well controlled particle size distribution (45 μm) which ensures safe deposition in the nasal cavity, whereas starch powder has a broader distribution with risk of deposition of starch powder in the lungs.

Whereas the bile acids taurocholate and desoxycholate are hemolytic, DSM produced no increase in erythrocyte hemolysis when incubated with rabbit erythrocytes. Although bile acids and DSM both enhance the absorption of insulin, DSMs can be considered more biocompatible.

To establish the safety of DSM, as a nasal drug delivery system toxicological and morphological studies on the nasal mucosa after repeated administration of spheres must be undertaken.

The present study indicates that DSMs and starch powders promote the absorption of insulin in the rat. The system must be insoluble in water but should absorb water. The water uptake may be critical for the mechanism of action. Presumably DSMs, when administered intranasally in dry form, adhere to the mucous membrane and start to swell, drawing water from the mucus and the underlying epithelial cells. This dehydration might induce reversible shrinking of the epithelial cells and widening of the tight junctions, thereby enhancing the transport of hydrophilic components such as insulin.

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