Journal of Controlled Release, 21 (1992) 165–172

COREL 00753

Microspheres as a nasal delivery system for peptide drugs

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(Received 7 July 1991; accepted in revised form 9 March 1992)

Microspheres of starch and dextran, cross-linked with epichlorohydrine, function as an enhancer system for the absorption of insulin in rats. The effect on the glucose level is rapid and maximal reduction of plasma glucose is seen within 30–40 min. Starch microspheres are more effective than dextran spheres in inducing a decrease in blood sugar. The starch microspheres have been evaluated from a toxicological point of view in rabbits. The spheres were administered 2 times per day for 8 weeks and in two dosages, 10 and 20 mg. Scanning electron microscopy of the nasal mucosa showed no alterations. The only finding observed in light microscopy was a small hyperplasia in the septum wall. A preliminary test on healthy volunteers with starch microspheres given nasally for 1 week shows good acceptability. A temporary widening of the tight junctions in a monolayer of human epithelial (Caco-2) cells was seen in the presence of dry starch microspheres. The widening of the tight junctions coincided with the increased absorption rate of insulin. A conceivable hypothesis with regard to the mechanism of action of DSM can be that the epithelial mucosa is dehydrated, with a reversible "shrinkage" of the cells, thus giving a physical separation of the intercellular junctions.

Key words: Insulin absorption; Microspheres

Introduction

The idea of giving peptides and proteins nasally is not new. Immediately after the discovery of insulin, 70 years ago, attempts were made to give insulin by the nasal route [1]. Since then, several peptides have been administered intranasally. In the treatment of diabetes insipidus, the nasal administration of vasopressin or its analogues is well known [2,3]. Some of the reasons why nasal administration is attractive are (a) rapid absorption and quick pharmacodynamic effect of the drug; (b) avoidance of first pass metabolism; (c) an easy administration route, particularly suitable for selfmedication.

However, even though the advantages of intranasal administration are clear, there are disadvantages, such as different drug distribution in the nose, giving variable absorption, low permeability of the peptide through the mucosa [4] and degradation of the peptide drug by proteolytic enzymes in the nose [5]. This often results in a low bioavailability of nasally given peptides. Consequently, a great deal of effort has been put into facilitating peptide absorption. Variations of pH [6], ionic strength [7], and inhibition of proteolytic enzymes in the nose [8] are some ex-

Often these steps are insufficient to achieve an acceptable bioavailability of the peptide. An enhancer is therefore usually needed to reach an acceptable absorption of larger peptides.

Several enhancers have been tested, such as surfactants [9], bile salts [10], gels [11] and fusidic acid derivatives [12]. When working with enhancers giving increased nasal absorption of peptides, one must carefully address the question of nasal irritation and cell damage. Recently, microspheres given as a nasal powder have been shown to promote the absorption of peptides/proteins [13]. The enhancement of drug absorption is obtained if the spheres are water-insoluble and able to take up water and swell [14].

This paper will give a survey of the use of different microspheres as a nasal absorption promoting system. Not only the technical aspects but also biological/clinical and toxicological issues regarding microspheres will be considered.

In vitro

Characteristics

The particle systems evaluated in this paper are based on starch and dextran cross-linked with epichlorohydrine. The degradable starch microspheres (DSM) are degradable with α-amylase whereas Sephadex and DEAE-Sephadex are non degradable in biological compartments such as the nasal cavity. DEAE-Sephadex microspheres are substituted with 2-ethylaminoethyl groups giving anion-exchange properties. All three particle systems fulfil the prerequisite for absorption enhancing properties, i.e. they are water-absorptive and water-insoluble.

According to the manufacturer, the dextran spheres will exclude all molecules with a molecular weight higher than 5,000. Consequently, insulin will not be incorporated in the spheres, but will instead be situated on the surface. The starch microspheres (DSM) have a cut-off at approximately 30,000–50,000, so insulin can in this case be situated both on the surface and inside the size. The starch spheres have a mean particle size of 45 μm whereas the dextran spheres have a particle size of between 50 and 180 μm.

Preparation of spheres

The dextran spheres and the degradable starch microspheres were mixed with human monocomponent insulin 100 IU/ml in a ratio of 100 mg spheres per ml insulin solution and 100 mg spheres per 800 μl insulin solution respectively. The obtained gels were freeze dried. The dry dextran spheres were passed through a 180 μm sieve and the starch powder through a 63 μm sieve.

DEAE-sephadex was suspended in a buffer solution to equilibrate the ion-exchange groups. After sedimentation the gel that had been formed was mixed with insulin and buffer. The suspension was again allowed to sediment and the gel formed was freeze dried. The dried spheres were passed through a 180 μm sieve.

Insulin release

The release kinetics were measured by placing 10 mg of the different sphere preparations in a diffusion chamber. The receiving compartment contained 0.15 M or 0.86 M sodium chloride solution. The experiments were performed as reported earlier [14]. The protein content was determined with the Folin-Lowry assay [15].

Starch microspheres with different swelling factors, i.e. different degrees of cross-linking, release insulin at different rates. The swelling factor, defined as the bead volume in cm^3 obtained when 1 g DSM is allowed to swell in a buffer, is low when the degree of cross-linking is high. The swelling of the spheres and the release of insulin from the spheres with the low swelling factor of 5 is rapid: 90% of the incorporated amount is released within 10 min. The rate of insulin release from loosely cross-linked starch with a swelling factor of 17 is slower: 60% of the incorporated amount is released within 10 min. The high swelling factor indicates that more liquid is needed for the spheres to be completely swollen.

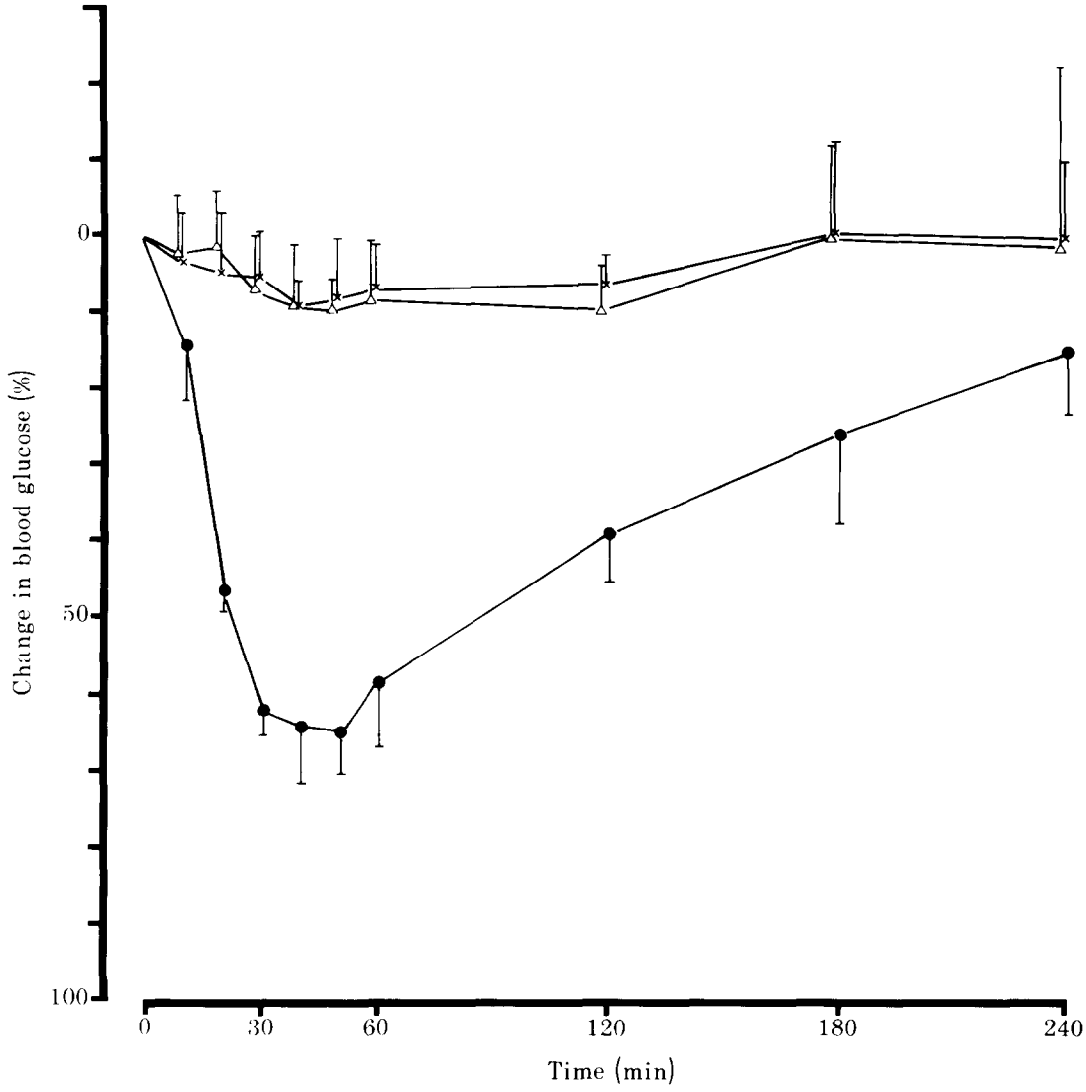

Fig. 1. Change in blood glucose in rats after intranasal administration of insulin. Empty DSM 0.5 mg/kg (×); soluble insulin 2.0 IU/kg (△); DSM + insulin 1.7 IU/kg (●). The data are expressed as mean ± SD (n=6–8). (From Björk and Edman [13], reproduced by permission of Elsevier.)

sulin to be dissolved and to diffuse out of the matrix [14].

The swelling factor of the dextran particles (Sephadex) is 4–6, i.e. more or less the same as the starch microspheres with a high degree of cross-linking. The release rate of insulin is also the same: 90% of the incorporated amount is released within 10 min. DEAE-Sephadex requires partment before the insulin is released. This suggests that the insulin is bound to the DEAE-groups and can only be displaced by high ionic strength [16].

In vivo

Biological effect

TABLE 1

Total decrease (D%) and maximal decrease of plasma glucose level after intranasal administration of insulin in three different sphere systems with the same swelling factor

	Dose (IU/kg)	D (%)	Maximal decrease (%)	Number of animals
DSM	1	16.4	26	6
Sephadex	1	7.5	24	3
DEAE-Sephadex	1	3.1	8	3

delivery studies. Three main models, with minor modifications, have been described in the literature [17]. The model used in these experiments is a modification of the "in vivo surgical model". Instead of preventing drainage of the drug from the nasal cavity by sealing the nasopalatine tract leading from the nasal cavity to the oral cavity with an adhesive glue [18], the normal function was maintained to the fullest possible extent.

The rats were anesthetized and operated on as earlier reported, [13]. The insulin preparations were given through the nostril with a polyethylene tube 30 min post-operation. The spheres were weighed in the tube and administered by blowing air from a syringe through the tube.

Insulin was administered in DSM (swelling factor 8–10) with an amount of 3.5 mg DSM/kg. This resulted in a rapid decrease in blood glucose (Fig. 1). The maximal reduction of the blood glucose level after a dose of 1.7 IU/kg body weight was 64%. The maximal decrease was reached approximately 40 min after administration and the glucose level was normalized after 4 h. The decrease in blood glucose was found to be dose-dependent. However, the time to maximal decrease and the time for the glucose level to normalize were independent of the dose [13]. A later study by Björk and Edman [14] showed that starch microspheres with different swelling factors are biologically equivalent in reducing the blood glucose level, in spite of the differences in in vitro release rate.

Insulin 1 IU/kg administered together with Sephadex particles, 5 mg/kg, also induced a rapid tent as DSM. The maximal decrease in plasma glucose, 25%, was seen 40–60 min after administration and the level was normalized within 3 h. DEAE-Sephadex, on the other hand had no effect on the glucose level. The total decrease in plasma glucose level (D%) was calculated according to Hirai:

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

AUC_c and AUC_i = area under plasma glucose vs time curve from 0 to 4 h after administration of control and insulin preparation. The total decrease values (D%) and the maximal decrease are summarized in Table 1.

A plausible explanation of the lack of effect with DEAE-Sephadex can be that the available ionic strength in the nasal cavity is too low to displace the bound insulin. Therefore saline of high ionic strength was administered nasally 5 min after instillation of the powder. However, no effect on the plasma glucose level was seen [16].

Insulin must probably be available for absorption during the first minutes after administration of the microspheres to have any effect on the blood glucose, i.e. during the time the spheres will absorb water and swell to a gel. This result supports the hypothesis launched by Edman and Björk [14] that during the time they swell the spheres will draw water from mucus and the underlying epithelial barrier and thereby cause widening of the tight junctions, resulting in enhanced transport of hydrophilic molecules.

Toxicological evaluation

The effect of DSM on the nasal mucosa after repeated administration was studied in rabbits [19]. Degradable starch microspheres (DSM) were administered twice daily for two, four or eight weeks. Two different doses were used, 10 and 20 mg. The spheres were administered with an insufflator which gave a good spreading of the

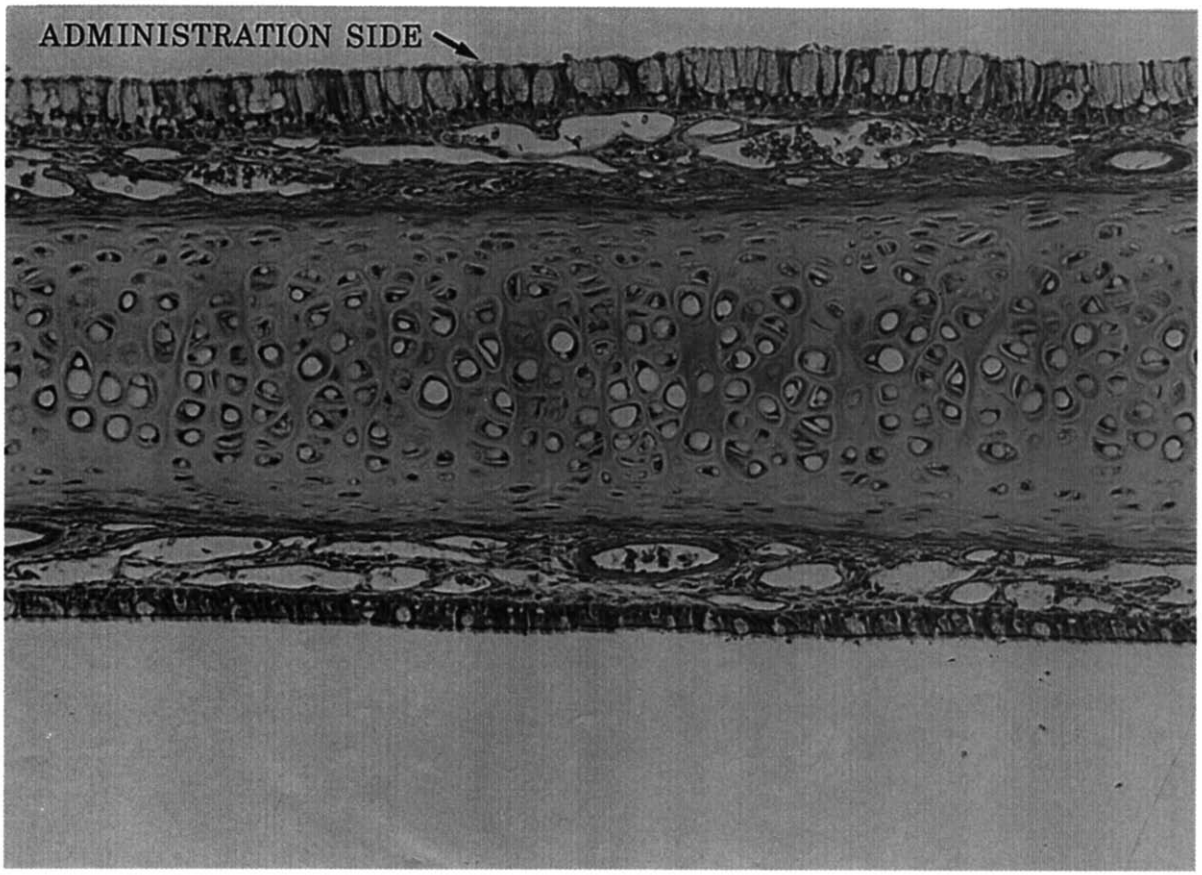

Fig. 2. Cross-section of the septum of one rabbit administered 20 mg DSM for 8 weeks. Mild hyperplasia of the columnar epithelium with an increased number of goblet cells on the administration side LM×200 (From Björk et al. [19], reproduced by permission of Elsevier).

nostril was used for dosing, the other served as a control. After the end of the administration period the noses were prepared for either scanning electron microscopy (SEM) or light microscopy (LM) study. The cilia were intact in all groups and no changes in the amount and location of the ciliated cells were seen with SEM. Only in some animals given DSM for 8 weeks did the nasal septum show mild, focal hyperplasia of the epithelium. It was characterized by a mild increase in the number of goblet cells and mild hyperplasia of the columnar epithelium (Fig. 2). The effect was more obvious with the higher dose. This is probably due to the water holding capacity of the microspheres giving a desiccated

Interaction with cells

The cell model (Caco-2), originating from a human colorectal carcinoma, was used for the permeability studies [20]. The cell model is a human intestinal epithelial cell line that differentiates to enterocyte-like cells under cell culture conditions.

The cells are grown on a special filter in a chamber allowing measurement of the transport of different marker molecules across the cell layer. In this model we have applied DSM on the top of the cells for a certain time and after that time the test substance ^{3}H-mannitol (MW 180)

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