ELSEVIER

International Journal of Pharmaceutics 125 (1995) 205–213

international journal of pharmaceutics

Influence of osmolarity on nasal absorption of insulin from the thermogelling polymer ethyl(hydroxyethyl)cellulose

Lena Pereswetoff-Morath [a,*], Peter Edman [a,b]

[a] *Department of Pharmacy, Uppsala University, S-751 23 Uppsala, Sweden*
[b] *Astra Hässle AB, S-431 83 Mölndal, Sweden*

Received 23 January 1995; revised 10 April 1995; accepted 12 April 1995

Abstract

Insulin, 3 IU/kg body weight, given intranasally in a hypoosmotic thermogelling system, containing EHEC, SDS, m-cresol and glycerol, is more efficient in lowering plasma glucose than insulin delivered in iso- and hyperosmotic gels. This has been shown for two osmotic agents, glycerol and creatinine. The effect is significantly different from a plain hypoosmotic insulin solution, which did not have any effect on the plasma glucose. This suggests that there is a synergistic effect of the hypoosmotic environment and the EHEC gel. However, both m-cresol and insulin participate in gel formation. Therefore, the insulin is partly associated with the gel matrix and not all of the insulin is available for absorption. An optimal gel system should be a thermosetting polymer solution with mucoadhesive properties, that rapidly releases the insulin content under hypoosmotic conditions.

Keywords: Nasal administration; Insulin; Thermogelling polymer; Osmolarity; Rheology

1. Introduction

The bioavailability of intranasally administered peptides is rather low and a number of ways to improve the absorption have been evaluated. Different types of absorption enhancers, such as anionic surfactants (Hirai et al., 1981), bile acid derivatives (Duchateau et al., 1986) and cyclodextrins (Schipper et al., 1990) have been tested. The increase in absorption shown for these systems may be due to a direct effect on the integrity of the cell membrane, inhibition of enzymatic activity in the nasal cavity or protection of the peptide from enzymatic degradation. Increased absorption of hydrophilic compounds may also be accomplished by manipulation of the tight junctional structure, e.g. alteration of extra- and intracellular calcium concentrations (Artursson and Magnusson, 1989), interference with the cytoskeleton (Madara, 1983), solvent drag phenomena (Pappenheimer and Reiss, 1987), or by exposing the epithelium to anisotonic solutions (Noach et al., 1994).

* Corresponding author.

Elsevier Science B.V.
SSDI 0378-5173(95)00124-7

The concept of increasing absorption by prolonging the contact time between the drug and the nasal mucosa is the basis for use of dry microspheres, which form a gel when they absorb water and thereby decrease the clearance from the nasal cavity (Illum et al., 1987). Prolonged contact time can also be achieved by adding polymers to a solution. There is a reversed correlation between viscoelastic properties and mucociliary transport rate, i.e. anionic polymers with high elastic and viscous modulus are cleared slowly from the nasal cavity (Lin et al., 1993). Various polymer solutions have proven successful in promoting nasal drug absorption (Potashnik et al., 1977; Morimoto et al., 1985, 1991). Viscous solutions have the disadvantage of being difficult to administer. Therefore, solutions of thermogelling polymers that have low viscosity at room temperature, which facilitates instillation into the nasal cavity, but which form a gel in situ in the nasal cavity when the temperature is raised, have been studied (Rydén and Edman, 1992). Ethylhydroxyethyl-cellulose (EHEC) and poly-*N*-isopropylacrylamide (NIPAAm) have a low critical solution temperatures (LCST) of approx. 32°C, i.e. the solubility of the polymers is dependent on the temperature. At temperatures above the LCST, EHEC will precipitate, whereas poly (NIPAAm) will shrink and release all incorporated water. In both cases, phase separation will occur. When a small amount of sodium dodecyl sulfate (SDS) is added to the non-ionic polymer EHEC, the cloud point is increased (Carlsson et al., 1986). For poly-*N*-isopropylacrylamide the same result can be achieved by co-polymerisation with acrylamide (Priest et al., 1987). Instead of phase separation at temperatures close to body temperature, highly viscous gels are created. The increase in viscosity is due to the formation of an expanding network that incorporates water. The network can be formed by the sodium dodecyl sulfate forming mixed micelles with the EHEC polymer chains (Carlsson et al., 1989a) and the acrylamide cross-linking the poly-*N*-isopropylacrylamide polymer chains (Priest et al., 1987). Therefore at a temperature of 37°C the systems will not separate, and homogenous gels are formed instead. When these systems were used as nasal drug delivery systems for insulin, the EHEC system produced a significant decrease in plasma glucose, whereas the NiPAAm system did not effect the glucose level. The difference between the two thermogelling systems was osmolarity, EHEC was hypoosmotic whereas the poly-NIPAAm system was isoosmotic (Rydén and Edman, 1992).

From the literature it is known that anisoosmotic solutions have an impact on cell volume, membrane resistance and transport of water, drugs or markers across different epithelia. A hypotonic solution caused hepatocytes to swell to adapt their intraosmotic pressure to the tonicity of surrounding medium. The swelling is followed by regulatory volume decrease (RVD) to return to their resting (isoosmotic) volume. In most cells RVD is due to net extrusion of K^+ and Cl^- with the accompanying loss of water (Corasanti et al., 1990). An apical hypoosmotic treatment of Caco-2 cell monolayers induced swelling of the cells but no RVD was observed. The observed enhanced transport of hydrophilic compounds through the paracellular pathway was believed to be due to asymmetric changes in cell volume originating from the lack of RVD. The transepithelial resistance (TEER) under the same conditions decreased during the period the treatment was applied (Noach et al., 1994). Pappenheimer and Reiss (1987) described an osmotic force for fluid absorption by sodium and glucose called solvent drag. Reduced osmolarities also produce an increase in net volume flow over gall bladder epithelia (Whittembury et al., 1980). Fluid flow from the hypotonic to the isotonic side of the cells will develop after application of a hypotonic solution, and hydrophilic solutes will be dragged along, i.e. solvent drag. Solvent drag through intercellular channels provides an effective mechanism for mass transport of hydrophilic solutes.

Exposure to hypertonic solutions made the cells shrink to bring the cytosol in osmotic equilibrium with the surrounding medium. The regulatory volume increase (RVI) that follows is due to ion and water entry into cells (Corasanti et al., 1990; Ritter et al., 1991). The transepithelial resistance increased when studies were performed on guinea pig small intestine (Madara, 1983) and on Madin-Darby canine kidney cells (Ritter et al.,

1991), whereas when the TEER was measured on Caco-2 cell monolayers the resistance was decreased (Noach et al., 1994).

The aim of this study was to evaluate the influence of osmolarity on the nasal absorption of insulin from a thermogelling polymer system. Further, the rheological properties of the gel system were studied to elucidate the influence of viscoelastic properties on the enhancing effect of the EHEC system.

2. Materials and methods

2.1. Materials

Ethyl(hydroxyethyl)cellulose (EHEC) of medical grade was obtained from Berol Nobel, Stenungsund, Sweden. Actrapid® was purchased from Novo, Denmark and human crystalline monocomponent insulin was obtained as a gift from the same company. Sodium dodecyl sulfate, specially pure, creatinine, m-cresol and glycerol were purchased from Sigma, USA. All other chemicals were of analytical grade.

2.2. Preparation of polymer solutions

0.85% EHEC, 2.6 mM sodium dodecyl sulfate and three different concentrations of glycerol or creatinine was mixed with Actrapid® (Novo, Denmark) in the proportion 7:3. The final concentration was 0.6% of EHEC, 1.8 mM sodium dodecyl sulfate, 30 IU/ml insulin, 0.9 mg/ml m-cresol and 4.8 mg/ml glycerol in all preparations. Insulin, m-cresol and glycerol originates from Actrapid®. There was additional glycerol (0, 2.6 or 5%) or creatinine (0.21, 2.65 or 4.70%) added to create hypo-, iso- and hyperosmolarity respectively (Table 1). All preparations had a pH of 7.4 ± 0.2.

2.3. Osmolarity measurements

Osmolarity of the polymer solutions was measured in a vapour pressure osmometer (5500 Vapor Pressure Osmometer, Vescor). A 10 μl sample is conveyed to the sample chamber, thermostated to 37°C. The dew point temperature depression is measured and registered by a microprocessor. All measurements were done in triplicate.

2.4. Rheology

From rheological experiments it is possible to conclude which ingredients participate in gel formation. An increase in elastic modulus (G') is evidence of a gel forming. The rheological properties were determined in a computer-controlled rheometer, Bohlin rheometer VOR. In non-de-

Table 1
Composition of thermogelling systems and their osmotic properties

Formulation	Osmotic agent	Osmotic pressure (mOsm)	Concentration					
			Osmotic agent (%)	EHEC (%)	SDS (mM)	Insulin (IU/ml)	m-Cresol (mg/ml)	Glycerol (mg/ml)
1. EHEC hypoosmotic	Gycerol	55	0.0	0.6	1.8	30	3.0	4.8
2. EHEC isoosmotic		387	2.6	0.6	1.8	30	3.0	4.8
3. EHEC hyperosmotic		668	5.0	0.6	1.8	30	3.0	4.8
4. Control hypoosmotic		103	0.0	–	–	30	3.0	4.8
5. Control isoosmotic		389	2.6	–	–	30	3.0	4.8
6. EHEC hypoosmotic	Creatinine	99	0.2	0.6	1.8	30	3.0	4.8
7. EHEC isoosmotic		282	2.6	0.6	1.8	30	3.0	4.8
8. EHEC hyperosmotic		458	4.7	0.6	1.8	30	3.0	4.8
9. Control hypoosmotic		103	0.2	–	–	30	3.0	4.8
10. Control hypoosmotic		100	0.2	–	1.8	30	3.0	4.8
11. Control hypoosmotic		100	0.2	0.6	–	30	3.0	4.8

structive oscillation experiments the response in stress of the viscoelastic material to a sinusoidally varying strain is monitored as a function of temperature. In the experiments a torsion bar of 1.66 g cm and the concentric cylinder system C14 with a sample volume of 3 ml was used. A oscillation frequency of 2 Hz and an amplitude of 30% was selected. The elastic (G') and viscous (G'') modulus was recorded five times at 20, 25, 30, 33, 35, 37 and 39°C. All samples were measured in triplicate.

2.5. Absorption study

Male rats, Lewis × DA F1-hybrids and Sprague-Dawley, weighing 250–300 g, were used. Anaesthesia and surgery were performed as earlier reported (Björk and Edman, 1988). Briefly, the animals were fasted for 15–17 h prior to the experiments. They were anaesthetised with intraperitoneal injection of 150 mg/kg thiobutabarbital sodium (Inaktin, BYK-Gulden) and placed in a supine position on heated plates to maintain body temperature. Trachea and arteria carotis were cannulated. 25–30 μl of the polymer solution was administered 30 min after surgery through the nostril to give an insulin dose of 3 IU/kg. Blood samples were withdrawn from the arteria carotis at intervals during four h.

After centrifugation, the plasma was withdrawn and frozen for glucose analysis by an enzymatic method catalysed by hexokinase and glucose-6-phosphate dehydrogenase in a Beckman Clinical System 700. Statistical significance was tested using the Student-Newman-Keul test.

3. Results

3.1. Osmolarity measurements

Addition of glycerol and creatinine rendered systems of different osmolarities, ranging from 55 mOsm to 668 mOsm. The osmolarities of the solutions used as controls in the in vivo absorption study corresponded to the osmolarities of the polymer solutions (Table 1).

3.2. Rheology

To investigate which ingredients in the polymer system that participate in the gelling process, the elastic modulus (G') was measured with different substances present (Fig. 1). The solution used was a hypoosmotic polymer system containing EHEC, SDS, glycerol, m-cresol and insulin. The concentrations correspond to Composition 1 used in the in vivo absorption study (Table 1). With all ingredients present there was a rapid increase in elastic modulus with increasing temperature, at 37°C the G' was 6.9 Pa compared with 0.1 Pa at 25°C. When insulin was excluded, the system still formed a gel but the G′ at 37°C was lower, 3.2 Pa. When also m-cresol was excluded and only EHEC, SDS and glycerol remained, the system had lost its gelling capacity and the elastic modulus was 0.1 Pa both at 37°C and at 25°C. There was no further decrease in G' when glycerol was excluded from the formulation.

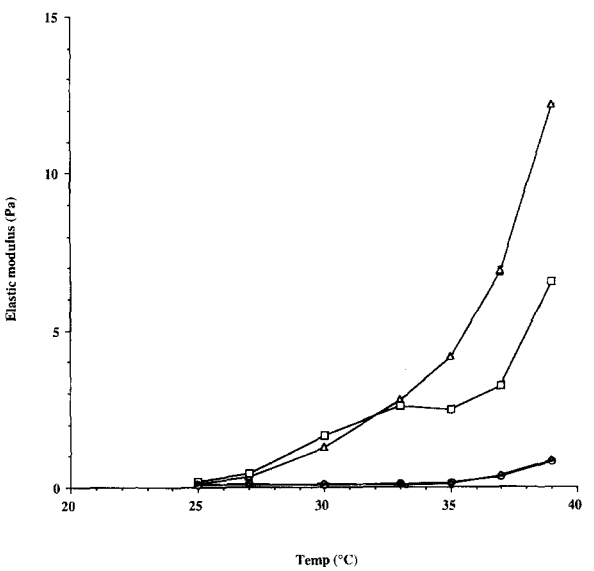

Fig. 1. The temperature dependence of the elastic modulus for polymer systems with different ingredients. 0.6% EHEC and 1.8 mM SDS are present in all solutions. To this have been added the ingredients of Actrapid®. (△) 30 IU/ml insulin, 0.9 mg/ml m-cresol and 4.8 mg/ml glycerol. (□) 0.9 mg/ml m-cresol and 4.8 mg/ml glycerol. (◇) 4.8 mg/ml glycerol. (○) No addition of Actrapid® related ingredients, i.e. contains only 0.6% EHEC and 1.8 mM SDS. Data are expressed as mean ± S.E.M.

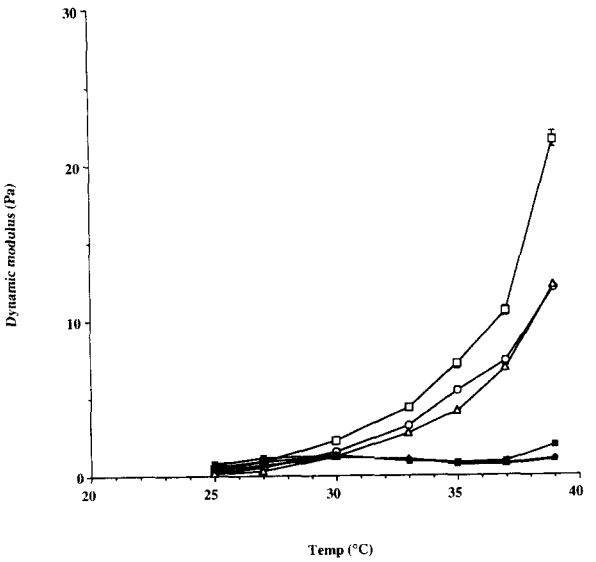

Fig. 2. Rheological properties of (△) hypoosmotic, (○) isoosmotic and (□) hyperosmotic EHEC gels. Open symbols, elastic modulus (G'); closed symbols, viscous modulus (G'') (mean ± S.E.M.)

Addition of large amounts of glycerol to ensure a hyperosmotic solution (Composition 3, Table 1) influenced the elastic but not to the viscous modulus. A larger increase in elastic modulus was observed compared with no or small amounts of glycerol present (Fig. 2). A system containing 5% glycerol gave a G' of 10.6 Pa at 37°C, whereas 2.6% glycerol in the gel gives a G' of 7.4 Pa at the same temperature. With no addition of glycerol, the gel is just slightly less elastic than the 2.6% glycerol gel, i.e. 6.9 Pa. The effect is even more pronounced at 39°C.

The variance in these results is small and the standard error of the mean bars (S.E.) falls within the symbols. Therefore, when there is no overlap of the symbols, there is a significant difference ($p < 0.05$) between the systems in Figs. 1 and 2.

3.3. Absorption study

When glycerol was used as osmotic agent the hypoosmotic system induced lowering of the plasma glucose, whereas practically no decrease in plasma glucose level was obtained with the iso- and hypertonic systems (Fig. 3). Insulin 3 IU/kg in the hypoosmotic system (Composition 1, Table 1), i.e. no glycerol added, caused a rapid decrease in plasma glucose, with the maximum effect, 26.6%, reached after approx. 40 min. The decrease was significantly different ($p < 0.01$) from the effect of both the other two polymer systems (Compositions 2 and 3, Table 1) and the hypo- and isoosmotic controls (Compositions 4 and 5, Table 1) from 20 min up to an hour after administration. The results with creatinine as the osmotic agent confirmed these findings (Table 2). However, it should be noted that the significant difference observed between the hypoosmotic system and the other polymer systems and controls, appears 30–120 min after dosing.

Table 2
Changes in plasma glucose after intranasal administration of insulin 3 IU/kg in different delivery systems

Formulation decrease (%)	Osmotic agent (%)	Maximum	Time to maximun decrease (min)	Number of animals
1. EHEC hypoosmotic	Gycerol	26.6	40	5
2. EHEC isoosmotic		6.0	30	6
3. EHEC hyperosmotic		8.6	40	5
4. Control hypoosmotic		5.8	20	4
5. Control isoosmotic		3.1	30	5
6. EHEC hypoosmotic	Creatinine	30.9	60	6
7. EHEC isoosmotic		8.5	50	6
8. EHEC hyperosmotic		9.4	40	6
9. Control hypoosmotic		9.9	40	3
10. Control hypoosmotic		11.9	60	3
11. Control hypoosmotic		11.1	60	3

Insulin, 3 IU/kg, administered in a plain hypoosmotic solution (Composition 9, Table 1) had little effect on the plasma glucose (Fig. 4). The lowering of the plasma glucose level obtained by insulin delivered in hypoosmotic 1.8 mM SDS solution (Composition 10, Table 1) or hypoosmotic 0.6% EHEC solution (Composition 11, Table 1) was not significantly different from the result of the plain hypoosmotic solution. However, insulin delivered in a system containing both 0.6% EHEC and 1.8 mM SDS (Composition 6, Table 1) i.e. the hypoosmotic thermogelling system, differed from the controls where EHEC and SDS were given separately. The difference was significant ($p < 0.05$) from 30 min to 2 h after administration and the maximum decrease, 30.9%, was reached after 60 min.

The various hypoosmotic controls used in the study with creatinine as the osmotic agent, i.e. plain solution, 1.8 mM SDS and 0.6% EHEC, produced approx. 10% lowering in plasma glucose level. When these results were compared with the iso- and hypoosmotic controls used for glycerol as the osmotic agent, there was no significant difference.

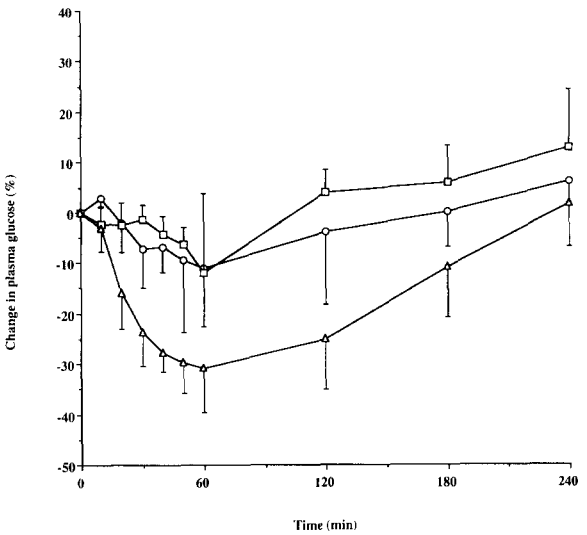

Fig. 4. Change in plasma glucose (mean ± S.D.) after intranasal administration of insulin, 3 IU/kg in a hypoosmotic gel, and hypoosmotic controls with creatinin as the osmotic agent. (△) Hypoosmotic gel; (○) control, hypoosmotic solution with 0.6% EHEC; (□) control, hypoosmotic solution with 1.8 mM SDS.

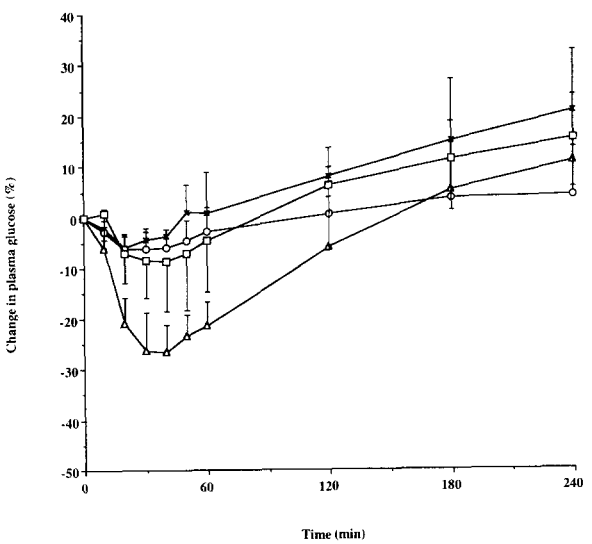

Fig. 3. Change in plasma glucose (mean ± S.D.) after intranasal administration of insulin 3 IU/kg in thermogelling EHEC gels with glycerol as the osmotic agent. (△) Hypoosmotic gel, (○) isoosmotic gel, (□) hyperosmotic gel and (×) control, plain hypoosmotic solution.

4. Discussion

In a previous work, when EHEC was used as a vehicle for insulin administration, it was only possible to incorporate 10 IU insulin per ml polymer solution (Rydén and Edman, 1992). At higher concentrations of insulin the polymer precipitated and phase separation occurred. To be able to increase the amount of insulin delivered to the animals, while maintaining a suitable volume for administration, it was essential to incorporate more insulin in the polymer system. When the amount of polymer and surfactant was decreased it was also possible to incorporate more insulin. The formulation chosen contained 0.6% EHEC, 1.8 mM SDS and 30 IU/ml insulin. Since insulin is added in the form of a commercially available

monocomponent insulin for injection, Actrapid®, there is also an addition of the excipients in Actrapid®. Consequently, there is 4.8 mg/ml glycerol and 0.9 mg/ml m-cresol present in all formulations tested.

From earlier works by Carlsson and colleagues (Carlsson et al. (1989a,b, 1990) it is known that SDS participates in the gelling process of EHEC by acting as bridges in the expanding network of polymer chains. The polymer, which is a relatively hydrophobic cellulose ether, has many hydroxyl groups. Theoretically, both hydrogen bonds and hydrophobic interactions may occur in the network. Insulin is a relatively large molecule, negatively charged at pH 7.4 due to being a zwitterion. These properties could favour accumulation of insulin to the mixed micelles of SDS on the polymer chains and thereby facilitate hydrogen bonding between insulin and EHEC. The preservative m-cresol, an aromatic alcohol, has the potential to establish both hydrogen bonds and hydrophobic interactions with EHEC. Glycerol is a hydrophilic aliphatic alcohol and may therefore only form hydrogen bonds with the polymer.

From the rheological experiments it was possible to conclude that both m-cresol and insulin participate in gel formation (Fig. 1). When these two ingredients are excluded the system loses its gelling capacity. This indicates that hydrogen bonds and hydrophobic interactions are necessary in this system to establish a gel matrix. Consequently, m-cresol and insulin are incorporated in the polymer matrix and will be released quite slowly. Preliminary data confirm that only 40% of the insulin is released from the hypoosmotic gel system during the first 60 min. This reduces the amount of insulin available for absorption, compared with insulin in microspheres where the insulin is immediately available for absorption (Björk and Edman, 1990; Rydén and Edman, 1992). This might be part of the explanation of the difference in efficacy between microspheres and gelling systems (Rydén and Edman, 1992).

Large amounts of glycerol give a more elastic gel than small or no addition of glycerol (Fig. 2). Probably the contribution to network stability of small amounts of glycerol is modest compared with the contributions of SDS, insulin and m-cresol. Therefore, there is just a small increase in elasticity for the system with 2.6% glycerol compared with the system without glycerol. Large amounts of glycerol, on the other hand, might compensate for the lack of hydrophobic interactions and thereby add more to the elasticity of the gel or perhaps stabilise the mixed micelles that acts like bridges in the network. Cheong and co-workers showed in 1992 that the higher the viscosity of a polymer system, the slower the release of the incorporated drug, if the release mechanism is diffusion. Preliminary data indicate that the hypertonic gel with large amounts of glycerol, consequently with higher viscosity, releases as little as 7% of the insulin in the first hour, i.e. a much slower release than the hypotonic gel.

In a previous study a comparison of different gelling systems such as hypotonic EHEC, isotonic poly-NIPAAm and dextran microspheres was made with respect to their capacity to promote absorption of insulin given nasally (Rydén and Edman, 1992). The kinetics of the plasma glucose vs. time curves were similar for all systems tested. This indicates that viscous polymer solutions and microspheres might have a similar mechanism of action. It has been shown that the absorption enhancing effect of microspheres is not only due to increased contact time between the spheres and nasal epithelium, but also to a direct effect on the tight junctions in the cell barrier (Björk et al., 1995). However, the maximum decrease in plasma glucose differed between the systems. Insulin 1 IU/kg in dextran microspheres gave a 25% decrease in plasma glucose, whereas the same dose administered in the EHEC system only gave a 12% lowering of the plasma glucose. The other thermogelling system tested, poly-*N*-isopropylacrylamide, had no effect on plasma glucose. The same degree of glucose lowering as with the EHEC system was obtained when 1 IU/kg insulin was delivered in a hypoosmotic 0.1% polyacrylic acid solution (Morimoto et al., 1985).

It is obvious from the study that the thermosetting gel system EHEC with SDS, insulin, m-cresol and glycerol in a hypoosmotic milieu

induces a rapid lowering of the plasma glucose level in rats, whereas hyper- or isotonic EHEC gels or plain hypotonic solutions with equivalent amounts of insulin have no significant effect on the plasma glucose. This synergistic effect of a hypoosmotic environment and EHEC is interesting, but the mechanism of action is not absolutely clear. The EHEC gel has mucoadhesive properties which may result in both prolonged residence time in the nasal cavity and a closer contact between the delivery system and the nasal epithelial cells. Consequently, insulin or other drugs that are mixed or dissolved in EHEC should be retarded in the nose with increased and closer contact to the mucosa, thereby producing improved conditions for absorption. A similar mechanism might be operating the synergistic effect seen for the polymer CMC Na and the absorption promoter sodium caprate when human epidermal growth factor is given both rectally and nasally (Murakami et al., 1991). Probably, when EHEC is given as a hypoosmotic solution, a gel is formed which attaches to the mucus layer and creates a temporary hypoosmotic situation close to the nasal epithelia. The hypoosmotic environment on the cell surface creates conditions that promote cell swelling (Corasanti et al., 1990) and thereby promoting absorption of hydrophilic compounds such as insulin (Noach et al., 1994). As the hypoosmotic gel has a limited buffering capacity, a rapid return to isoosmotic conditions should occur giving a pulsatile delivery of insulin. The plasma glucose curve (Fig. 3) provides confirmation of this. No prolonged effect is seen, and a normalised plasma glucose level is observed 120 min post-dosing. The pharmacokinetic profile of the curve is almost similar to an intravenous injection of insulin, which indicates a rapid and limited absorption.

In summary, the results of this study indicate that a gel system could be found that improves the nasal absorption of insulin. A thermosetting gel with mucoadhesive properties, where the polymer does not interact with insulin and rapidly releases the drug under hypoosmotic conditions, should give the best conditions for nasal absorption of insulin.

Acknowledgements

The authors wish to thank Dr. Anders Carlsson for sharing his knowledge of the complex thermogelling EHEC, and Ann-Charlotte Olsson for performing the RIA analysis. Novo, Denmark is kindly acknowledged for their gift of human crystalline monocomponent insulin.

References

Artursson, P. and Magnusson, C., Epithelial transport of drugs in cell culture. II: Effect of extracellular calcium concentration on paracellular transport of drugs of different lipophilicities across monolayers of intestinal epithelial (Caco-2) cells. *J. Pharm. Sci.*, 79 (1989) 595–600.

Björk, E. and Edman, P., Degradable starch microspheres as a nasal delivery system for insulin. *Int. J. Pharm.*, 47 (1988) 233–238.

Björk, E. and Edman, P., Characterization of degradable starch microspheres as a nasal delivery system for drugs. *Int. J. Pharm.*, 62 (1990) 187–192.

Björk, E., Isaksson, U., Edman, P. and Artursson, P., Starch microspheres induce pulsatile delivery of drugs and peptides across the epithelial barrier by reversible separation of the tight junctions. *J. Drug Target.*, 2 (1995) 501–507.

Carlsson, A., Karlström, G. and Lindman, B., Synergistic surfactant –electrolyte effect in polymer solutions. *Langmuir*, 2 (1986) 536–537.

Carlsson, A., Karlström, G. and Lindman, B., Characterization of the interaction between a nonionic polymer and a cationic surfactant by Fourier transform NMR self-diffusion technique. *J. Phys. Chem.*, 93 (1989a) 3673–3677.

Carlsson, A., Lindman, B., Watanabe, T. and Shirahama, K., Polymer-surfactant interactions. Binding of N-tetradecylpyridinium bromide to ethyl(hydroxyethyl)cellulose. *Langmuir*, 5 (1989b) 1250–1252.

Carlsson, A., Karlström, G. and Lindman, B., Thermal gelation of nonionic cellulose ethers and ionic surfactants in water. *Colloids Surfact.*, 47 (1990) 147–165.

Cheong, L.W.S., Heng, P.W.S. and Wong, L.F., Relationhip between polymer viscosity and drug release from a matrix system. *Pharm. Res.*, 9 (1992) 1510–1514.

Corasanti, J.G., Gleeson, D. and Boyer, J.L., Effects of osmotic stresses on isolated rat hepatocytes. I. Ionic mechanisms of cell volume regulation. *Am. J. Physiol.*, 258 (1990) G290–G298.

Duchateau, G.S.M.J.E., Zuidema, J. and Merkus, F.W.H.M., Bile salts and intranasal drug absorption. *Int. J. Pharm.*, 31 (1986) 193–199.

Hirai, S., Yashiki, T. and Mima, H., Effect of surfactants on the nasal absorption of insulin in rats. *Int. J. Pharm.*, 9 (1981) 165–172.

Illum, L., Jørgensen, H., Bisgaard, H., Krogsgaard, O. and Rossing, N., Bioadhesive microspheres as a potential nasal drug delivery system. *Int. J. Pharm.*, 39 (1987) 189–199.

Lin, S.Y., Amidon, G.L., Weiner, N.D. and Goldberg, A.H., Viscoelasticity of anionic polymers and their mucociliary transport on the frog palate. *Pharm. Res.*, 10 (1993) 411–417.

Madara, J.L., Increases in guinea pig small intestinal transepithelial resistance induced by osmotic loads are accompanied by rapid alterations in absorptive-cell tight junction structure. *J. Cell Biol.*, 97 (1983) 125–136.

Morimoto, K., Morisaka, K. and Kamada, A., Enhancement of nasal absorption of insulin and calcitonin using polyacrylic acid gel. *J. Pharm. Pharmacol.*, 37 (1985) 134–136.

Morimoto, K., Yamaguchi, H., Iwakura, Y., Morisaka, K., Ohashi, Y. and Nakai, Y., Effects of viscous hyaluronate-sodium solutions on the nasal absorption of vasopressin and an analogue. *Pharm. Res.*, 8 (1991) 471–474.

Murakami, T., Kishimoto, M., Kawakita, H., Kisaki, M., Kojima, Y., Higashi, Y., Amagase, H., Fuwa, T. and Yata, N., Enhanced rectal and nasal absorption of human epidermal growth factor by combined use of the absorption promotor and the synthetic polymer in rats. *Eur. J. Drug Metab. Pharmacokinet.*, Spec. Issue 3 (1991) 125–131.

Noach, A.B.J., Sakai, M., Blom-Roosemalen, M.C.M., de Jonge, H.R., de Boer, A.G. and Breimer, D.D., Effect of anisotonic conditions on the transport of hydrophilic model compounds across monolayers of human colonic cell lines. *J. Pharmacol. Exp. Ther.*, 270 (1994) 1373–1380.

Pappenheimer, J.R. and Reiss, K.Z., Contribution of solvent drag through intercellular junctions to absorption of nutrients by the small intestine of the rat. *J. Membrane Biol*, 100 (1987) 123–136.

Potashnik, G., Ben-Adereth, N., Lunenfeld, B. and Rofe, C., Assessment of pituitary response to nasal application of synthetic gonadotropin-releasing hormone in men. *Fertil. Steril.*, 28 (1977) 650–654.

Priest, J.H., Murray, S.L., Nelson, R.J. and Hoffman, A.S., Lower critical solution temperatures of aqueous copolymers of N-isopropylacrylamide and other N-substituted acrylamids. In Russo, P. (Ed.), *Reversible Polymeric Gels and Related Systems. ACS Symposium Series*, American Chemical Society, Washington DC, 1987, pp. 255–264.

Ritter, M., Steidel, M. and Lang, F., Inhibition of ion conductances by osmotic shrinkage of Madin-Darby canine kidney cells. *Am J. Physiol.*, 261 (1991) C602–C607.

Rydén, L. and Edman, P., Effect of polymers and microspheres on the nasal absorption of insulin in rats. *Int. J. Pharm.*, 83 (1992) 1–10.

Schipper, N.G.M., Hermens, W.A.J.J., Romeyn, S.G., Verhoef, J. and Merkus, F.W.H.M., Nasal absorption of 17-beta-estradiol and progesterone from a dimethyl-cyclodextrin inclusion formulation in rats. *Int. J. Pharm.*, 64 (1990) 61–66.

Whittembury, G., de Martínez, C.V., Linares, H. and Paz-Aliaga, A., Solvent drag of large solutes indicates paracellular water flow in leaky epithelia. *Proc. R. Soc. Lond. B*, 211 (1980) 63–81.