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Lipid emulsions as vehicles for enhanced nasal delivery of insulin

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Abstract

The objective of this work is to explore lipid emulsion based formulations of insulin as an enhancer of nasal absorption. Insulin was incorporated into the aqueous phases of water-in-oil (w/o) and oil-in-water (o/w) emulsions. The formulations were perfused through the nasal cavity of rats in situ. Enhancement of insulin absorption was observed when insulin was incorporated into the continuous aqueous phase of an o/w emulsion. The presence of a small fraction of oil droplets along with insulin in the aqueous phase appeared to favor insulin absorption. When the oil phase constitutes the external phase, as in w/o emulsion, no insulin absorption was noted. Inhibition of insulin absorption might arise from a rate limiting barrier effect of the membrane completely covered by a stagnant oil layer. The in situ model was validated by in vivo experiments, which also revealed an increase in insulin absorption with o/w emulsions. However at lower insulin doses there was no statistically significant enhancing effect. In situ perfusion experiments across rat nasal pathway appear to be an appropriate model to study the enhancement effect of nasal formulations.

Keywords: Insulin; Nasal absorption; Lipid emulsion; Absorption enhancement; Systemic bioavailability

1. Introduction

Nasal administration may be a promising route for long term systemic delivery particularly when the drug is ineffective orally due to first-pass metabolism (Quraishi et al., 1997; Hussain, 1998). Small lipophilic molecules are generally well absorbed through the nasal mucosa. However the same is not true for high molecular weight hydrophilic peptides like insulin, which requires the use of absorption promoters. Many formulations incorporating amphiphilic molecules, i.e. bile salts (monomers or micelles) (Gordon et al., 1985; Shao and Mitra, 1992), fatty acids (Suzuki et al., 1998), bile salt/fatty acid mixed micelles (Tengamnuay and Mitra, 1990; Shao and Mitra, 1992), phospholipids (Drekker et al., 1992; Chandler et al., 1994), surfactants (Hirai et al., 1981a), cy-

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clodextrins (Merkus et al., 1991; Shao et al., 1992), and water soluble polymers or microspheres (Pereswetoff-Morath, 1998) have been studied as enhancers of insulin absorption. The mechanisms of absorption enhancement may involve many factors, i.e. a local alteration in the membrane structure and modification of its permeability (Hirai et al., 1981b; Martin et al., 1995), bioadhesion to the mucosal surface and lowering of the mucociliary clearance (Martin et al., 1998), opening of cellular tight junctions (Pereswetoff-Morath, 1998), oligomer dissociation (Shao et al., 1993) and/or the reduction in the permeant metabolism (Sakar, 1992; Shao and Mitra, 1992).

Most of the work found in the literature concerning emulsions of insulin deals with oral delivery using w/o emulsions (Tenktrog and Muller, 1995), microemulsions (Constantidines et al., 1994) or multiple emulsions (Silva Cuhna et al., 1997; Suzuki et al., 1998) in which the polypeptide is entrapped in a water core primarily for protection against intestinal proteases. To the authors' knowledge, emulsion systems have not been employed so far for nasal delivery purpose and in this article, nasal administration of insulin formulated in lipid emulsions is reported for the first time.

In situ rat nasal perfusion technique has been selected as an experimental model to study insulin absorption from lipid emulsion vehicles. This technique allows a continuous contact between the emulsion and the nasal epithelia through stirring and recirculation and generates reproducible results. Insulin, which has a very low water to oil partition coefficient (Banks et al., 1985), is solubilized in the aqueous phase of different types of emulsion. The peptide is either incorporated in the continuous phase of an oil-in-water (o/w) emulsion or entrapped in the aqueous core of a water-in-oil (w/o) emulsion. The in situ perfusion model has been validated by in vivo experiments, which also showed an increase in insulin absorption from o/w emulsion vehicle. Correlation between the emulsion physicochemical properties and nasal absorption of insulin has been attempted in order to achieve a better understanding of the enhancement mechanism.

2. Experimental section

2.1. Materials

The emulsifiers, polyoxyethylene 20 sorbitan monooleate (Tween 80®) and sorbitan monooleate (Span 80®) were obtained from Atlas Industries (Wilmington, DL). Soybean oil was purchased from Ruger Chemicals (Hillside, NJ). Isotonic phosphate buffer (pH 7.4) was used to prepare the aqueous phase of the emulsion. Zinc insulin (potency 26.3 U/mg) was obtained as a gift from Lilly Research Laboratory (Indianapolis, IN).

2.2. Insulin solution preparation and properties

Zinc insulin powder was dissolved in a few drops of 0.1 N hydrochloric acid solution to which isotonic phosphate buffer was added. The solution pH was adjusted to 7.4 by the addition of 0.1 N sodium hydroxide if necessary. The insulin concentrations used were 1 U/ml for the in situ perfusion experiments and 15 and 26 U/ml for the in vivo nasal absorption studies. These insulin solutions will represent the aqueous phase of various emulsion formulations.

2.3. Emulsion formulation

o/w and w/o emulsions of soybean oil (unsaturated vegetable oil) were prepared using a mixture of non-ionic emulsifiers (Tween 80® and Span 80®). According to a published report, the required HLB of the emulsifier mixture for o/w emulsions of soybean oil is 6 (Shinoda and Kunieda, 1983). It corresponds to a mixture of 84% Span 80® and 16% Tween 80® by weight.

The o/w emulsion composition (by weight) was 95% aqueous phase (insulin solution in isotonic phosphate buffer), 4.5% soybean oil and 0.5% of emulsifier mixture (5/95 o/w).

A w/o emulsion containing 20% aqueous phase, 72% soybean oil and 8% of emulsifier mixture (Span®/Tween® ratio 94/6) was also prepared (20/80 w/o).

Initially the emulsifiers were gently mixed with soybean oil at 60°C. Then the emulsions were obtained by mixing at room temperature the

aqueous and oil phases utilizing a rotor/stator type homogenizer (Tissue Tearor Model: 985-370, Biospec Products) at a high speed (about 10 000 r.p.m.) for 5 min.

The emulsions have been characterized immediately after their preparation by determining the emulsion type (o/w or w/o) and observing the droplet radius under the microscope (Carl Zeiss Telaval 31). A water-soluble dye (eosin) was added to the emulsion to examine the nature of the aqueous phase (continuous or disperse phase). Microscopic observation showed that the 5/95 o/w emulsion was characterized by an homogeneous distribution of small globules of radius below 4 μm. The 20/80 w/o emulsion had a broader distribution of droplet radii from below 1–10 μm. Both emulsions were stable during the time course of an experiment (from microscopic observations).

The effect of high shear on the insulin molecule conformation was studied by circular dichroism (CD) (spectropolarimeter JASCO J/720, Japan Spectroscopic Co.).

To study the effect of the surfactants alone, a control system containing 99.5% aqueous phase and 0.5% surfactant mixture was prepared by homogenization, according to the same protocol described earlier for the emulsion preparation.

2.4. Nasal perfusion of insulin emulsions

To study the nasal absorption of drugs in an animal model such as rodents, an in situ rat nasal perfusion technique can be utilized (Hussain, 1998). In this procedure, the formulation containing the drug is perfused through the rat nasal cavity in a recirculating fashion. The drug concentration remaining in the perfusate is measured as a function of time. This experiment allows for the determination of the drug absorption kinetics through the nasal mucosa (Hirai et al., 1981c; Huang et al., 1985; Shao et al., 1992). With nasal perfusion of emulsion, the analysis of the drug in the perfusate is difficult since it requires breaking phase boundary of the emulsion followed by an analysis of the aqueous phase.

In this study, a continuous perfusion of emulsion containing insulin through the nasal cavity of the rat was performed using an in situ procedure and blood samples were periodically withdrawn from the jugular vein to study the kinetics of absorption of insulin into the systemic circulation (pharmacokinetic measurement).

Hirai and Huang (Hirai et al., 1981c; Huang et al., 1985) developed the in situ rat nasal perfusion technique. These authors have published details of the surgical procedure. Briefly, male Sprague–Dawley rats weighing 270–300 g were fasted for 15–20 h prior to an experiment, but water was allowed ad libitum. The rats were anesthetized by an intraperitonal injection of ketamine/xylazine (9:1) solution (100 mg/kg). A polyethylene tube (PE-200, Intramedic, Clay Adams, NY) was then inserted into the trachea to maintain respiration. Another tube (PE-50) was inserted through the esophagus toward the posterior part of the nasal cavity. The nasopalatine tract was then sealed with an adhesive agent (Instant Jet, Carl Goldberg Models, Chicago, IL) to prevent the drainage of perfused emulsion into the mouth. Twenty ml of emulsion containing insulin was placed in a beaker maintained at 37°C and continuously stirred. A funnel was placed between the nose of the animal and the beaker in order to drain the perfusate from the nostrils back into the reservoir. The emulsion was recirculated through the nasal cavity of the rat through a peristaltic pump at a rate of 2 ml/min for up to 3–4 h. Blood samples (0.3 ml each) were withdrawn simultaneously via the jugular vein at 0, 5, 10, 20, 30, 60, 90, 120, 150, and 180 min following the onset of the perfusion. The samples were collected in heparanized centrifuged tubes (Fisher Scientific products) and centrifuged at 10 000 rpm for 10 min at 4°C (Beckman TL-100 ultracentrifuge). Plasma samples were isolated and kept frozen until further analysis.

A control perfusion experiment was also performed with an identical insulin concentration in buffer (1 U/ml). At least three experiments (each one with a different rat) were performed for each emulsion formulation. Statistical comparisons between the various treatments were made by Student's T test.

2.5. In vivo nasal absorption of insulin from emulsion

The in vivo model is a classical technique used to study the nasal absorption of drugs into the systemic circulation (Hirai et al., 1981c). In this technique, a small volume of drug preparation is directly placed into the rat nostril.

Briefly, male Sprague–Dawley rats weighing 270–300 g were fasted for at least 15–20 h prior to an experiment but water was allowed at libitum. The rats were anesthetized with sodium pentobarbital. The surgical procedure (Hirai et al., 1981c) is similar to that for the in situ procedure, except that the polyethylene tube inserted into the esophagus was closed at the end by an adhesive agent to prevent the drug loss due to esophageal drainage. After surgery, 100 μl insulin preparation was administrated to the nasal cavity through one of the nostrils via a microsyringe attached to a blunt needle at the end of which was 0.5 in. long PE-50 polyethylene tubing. The nostril was then closed immediately with an adhesive agent. Insulin doses administrated were 1.5 and 2.6 U for a 300-g rat, corresponding to 5 and 8.7 U/kg body weight. To achieve the specified dose and volume, insulin concentrations in the preparations (5/95 o/w emulsion and control experiments) were adjusted to 15 and 26 U/ml, respectively. To study the effect of the surfactants alone two different control systems were used: one with pure buffer and one with 0.5% emulsifier mixture.

Blood was withdrawn from the jugular vein and plasma samples were processed by the same procedure described for the in situ experiment.

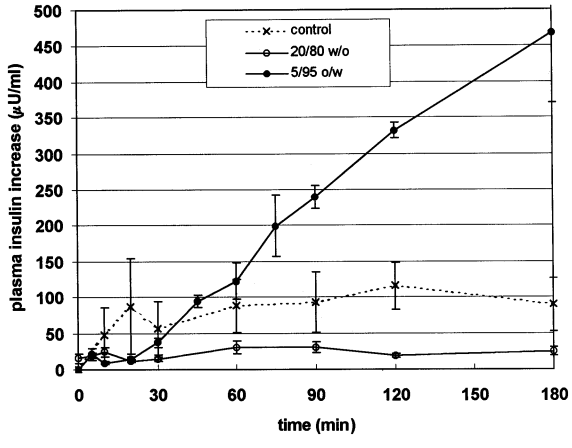

Fig. 1. Plots of plasma insulin concentration increase (μU/ml) vs. time during the continuous nasal perfusion of insulin formulated in emulsions and in buffer. Insulin concentration in the emulsion aqueous phase and in buffer control: 1 U/ml. (×, buffer control; ○, 20/80 w/o emulsion; ●, 5/95 o/w emulsion). Values denote means ± S.E.

2.6. Plasma insulin determination

Plasma immunoreactive insulin was quantitated by a double-antibody radioimmunoassay using RIA kit provided by Diagnostics Products Corporation, Los Angeles, CA.

3. Results

3.1. Systemic insulin absorption from the nasal perfusion

The kinetics of insulin absorption during continuous nasal perfusion of insulin in o/w emulsion has been illustrated by a gradual increase in plasma insulin concentration as a function of time (Fig. 1). Average values with the S.E.M. were plotted for each type of emulsion formulation to compare the absorption efficiencies. The areas under the curve (AUC) were calculated after subtraction of the basal plasma insulin concentrations obtained at time zero.

From a buffer control, insulin concentration was slightly elevated and reached a plateau at a value close to 100 μU/ml within about 20 min of perfusion. When emulsions are perfused, insulin absorption appears to depend on the nature of the emulsion (w/o or o/w). In case of w/o emulsion, insulin blood levels do not appear to rise significantly and remain fairly low in comparison to the control, around 25 μU/ml. The AUC values obtained are not significantly different than for the buffer ($P > 0.05$). These results indicate that the oil phase probably constitutes an additional barrier, which prevents the partitioning of insulin into the continuous oil phase thereby limiting its uptake into the nasal mucosal epithelium.

Table 1
Pharmacokinetic parameters of insulin administrated by nasal continuous perfusion[a]

Formulation	n[b]	C[c] (μU/ml) $t = 180$ min	$AUC_{0-120min}$ (μU/ml.min)	$AUC_{0-180min}$ (μU/ml.min)
Insulin in buffer-control	3	90 ± 40	9680 ± 4630	$15\,845 \pm 6730$
5/95 o/w emulsion	3	470 ± 90	$17\,390 \pm 1810$	$41\,390 \pm 5010$
20/80 w/o emulsion	3	10 ± 5	1050 ± 720	1440 ± 1000

[a] Values denote means ± S.E.
[b] n is the number of determinations.
[c] C is the plasma blood insulin increase obtained after 3 h of continuous perfusion. AUC, area under curve of insulin concentration versus time calculated after 2 and 3 h of continuous perfusion.

On the contrary, the absorption of insulin is enhanced when the polypeptide is incorporated in the continuous aqueous phase of the o/w emulsion. Insulin concentration slowly increased and reached a value of 470 μU/ml after 3 h. The area under the plasma insulin concentration–time curve (AUC) during the 180 min period increased from 15 845 μU/ml.min for insulin in buffer control compared to 41 390 μU/ml.min for insulin in o/w emulsion, value which is significantly greater ($P < 0.05$).

Table 1 summarizes the pharmacokinetic parameters obtained during the continuous perfusion of insulin emulsions.

3.2. Insulin absorption in vivo

As shown in Fig. 2, insulin administrated in o/w emulsion at a dose of 5 U/kg did not result in a significant change in insulin absorption. The nasal administration of 5 and 8.7 U/kg insulin dissolved in buffer or in the 0.5% emulsifier mixture resulted in a small elevation of plasma insulin level which reached a plateau around or below 100 μU/ml, similarly to the in situ experiment. Their corresponding AUC values are not significantly different ($P > 0.05$). However, when formulated in o/w emulsion at a dose of 8.7 U/kg, a rapid absorption of insulin in systemic circulation was observed, reaching a maximum plasma level of 360 μU/ml at 10 min. The area under the plasma insulin concentration-time curve during a 120 min period increased from 5800 μU/ml.min for insulin with the 0.5% emulsifier mixture control to 21 280 μU/ml.min with the o/w emulsion vehicle. The AUC values obtained during 90 and 120 min periods with the o/w emulsion are significantly higher than with the control experiments ($P < 0.05$). Table 2 summarizes the pharmacokinetic parameters obtained following the in vivo administration of insulin emulsions.

4. Discussion

The results presented in this article tend to suggest that the nasal absorption of insulin in o/w vehicles is significantly enhanced in both in situ and in vivo animal models. However at a lower

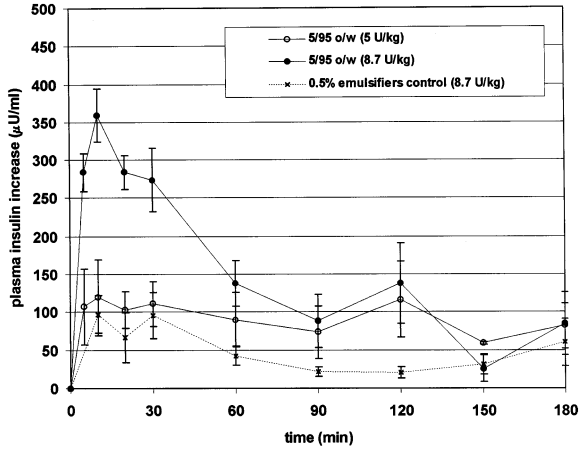

Fig. 2. Plots of plasma insulin concentration increase (μU/ml) vs. time following the in vivo administration of insulin formulated in emulsion and in 0.5% emulsifier mixture in buffer. Insulin doses: 8.7 U/kg (×, 0.5% emulsifiers in buffer; ●, 5/95 o/w emulsion) and 5 U/kg (○, 5/95 o/w emulsion). Values denote means ± S.E.

Table 2
Pharmacokinetic parameters of insulin administrated by the in vivo technique[a]

Formulation	Insulin dose (U/kg)	n[b]	C[c] (μU/ml)	$AUC_{0-90min}$ (μU/ml.min)	$AUC_{0-120min}$ (μU/ml.min)
Insulin in buffer-control	5	3	140 ± 60	4545 ± 3280	7520 ± 4820
5/95 o/w emulsion	5	4	120 ± 40	8480 ± 3165	11 330 ± 4520
Insulin in buffer-control	8.7	4	115 ± 50	7010 ± 2810	8330 ± 3570
Insulin in 0.5% emulsifier control	8.7	4	97 ± 30	5165 ± 1660	5800 ± 1850
5/95 o/w emulsion	8.7	3	360 ± 35	17 890 ± 2870	21 280 ± 4190

[a] Values denote means ± S.E.
[b] n is the number of determinations.
[c] C is the maximum increase of plasma blood insulin following in vivo administration. AUC, area under curve of insulin concentration versus time calculated 1 h 30 min and 2 h after the nasal administration of insulin preparation.

insulin dose of 5 U/ml there was no statistically significant enhancing effect. The emulsifier mixture alone did not induce any enhancement of in vivo insulin absorption. The w/o emulsion containing 8% emulsifiers completely inhibited insulin in situ absorption, a clear evidence that the emulsifier is not modulating the absorption enhancement effect. Previous report showed that Tween 80® alone has only a small effect on the pulmonary absorption of insulin (Li et al., 1993). Tween 80® and Span 80® are expected to have very little effect on the membrane integrity (Hirai et al., 1981a). Moreover, the emulsifiers employed in this study, as it has been shown before for Tween 80®, are unable to dissociate insulin oligomers, and this mechanism is unlikely to contribute to the absorption enhancement (Shao et al., 1993). The effect of high shear during emulsion formulation on insulin conformation was studied by CD spectroscopy. Fig. 3 first shows that the conformation of insulin molecules in the 26 U/ml aqueous solution is hexameric, as expected from previous report (Shao et al., 1993). This conformation was not modified by the homogenization conditions. HPLC experiments confirmed that no chemical degradation has occurred during the mixing (data not shown).

Insulin solutions prepared for in vivo and in situ studies fall within different concentration ranges, in which insulin molecules reside in different states of aggregation. Insulin molecules associate in aqueous solutions mainly to dimers and hexamers with two coordinated Zn^{2+} ions (Hvidt, 1991). The corresponding association constants in neutral solutions were estimated by Hvidt (Hvidt, 1991) and their values allowed us to calculate the amount of the different oligomers as a function of the total insulin concentration (Fig. 4). It shows that at 15 and 26 U/ml, hexamer is the major oligomer (this was verified by circular dichroism). At a concentration of 1 U/ml, monomers, dimers and hexamers are all present in similar amounts. Despite the differences in oligomer distribution, the absorption of both systems was enhanced in the presence of an o/w emulsion vehicle.

When emulsions are used as nasal formulations, the interaction between the oil droplets and the nasal mucosal membrane is likely to play an important role in the enhancement mechanism. A

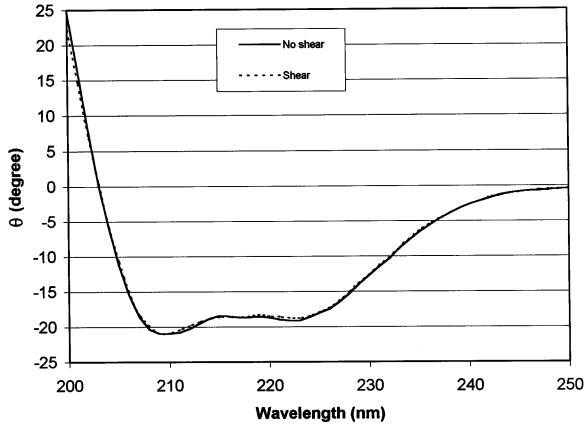

Fig. 3. Circular dichroism spectra of 26 U/ml insulin solutions prior to and following high shear.

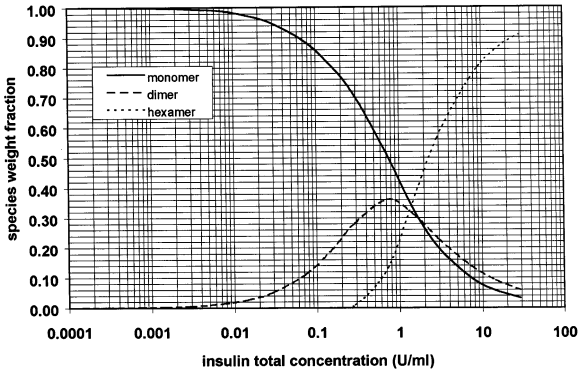

Fig. 4. The weight fraction of the insulin monomer, dimer and hexamer as a function of the total insulin concentration. The values of the dimer and hexamer dissociation equilibria constants employed to construct the diagram are respectively $K_{1,2} = 14 \times 10^4\ M^{-1}$ and $K_{2,6} = 15 \times 10^{10}\ M^{-2}$ (Hvidt, 1991).

previous study from the laboratory reported pulmonary absorption enhancement of insulin in the presence of liposomes (Liu et al., 1993). A similar uptake phenomenon and pharmacodynamic results were obtained when insulin was entrapped in the liposomes or when insulin was incorporated within the continuous aqueous phase of blank liposome dispersion. It was pointed out that insulin molecules, which are amphiphilic in nature, probably adsorb at the surface of the liposomes, as reported previously (Wiessner and Hwang, 1982). Similarly, insulin may adsorb at the surface of the emulsion droplets and interact with the emulsifier layer. However at lower insulin doses there was no statistically enhancing effects. The adhesion of the oil droplets at the mucosal membrane will then induce an accumulation of insulin at the membrane-aqueous interface. However, the number of droplets in contact with the surface must be controlled in order to avoid the formation of an oil layer at the membrane interface. A similar effect was observed with cholesterol used as an absorption enhancer (Mouristen et al., 1995). For low amounts, an increase in the permeability was observed, whereas for higher amounts, a decrease in the permeability was observed which revealed a sealing effect of the membrane interface due to an accumulation of cholesterol molecules.

Emulsions containing a small oil phase fraction (5%) appear to be promising formulations for nasal delivery of insulin. Low oil fraction is likely to cause minimal adherence to the mucosal surface compared to surfactant solutions. More work is needed to optimize the emulsion formulation and to study the biological effects of the different emulsion components. Future studies will also include an investigation of the interactions between insulin molecules and the oil droplets, as well as transport experiments with insulin containing emulsions across liquid and in vitro cell-culture biomembranes.

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