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In vitro study of polyoxyethylene alkyl ether niosomes for delivery of insulin

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Abstract

In this study, niosomes of polyoxyethylene alkyl ethers (Brij^{TM}) were prepared for encapsulation of insulin by film hydration method. Without cholesterol, brij 35 and brij 58 did not form niosomes, apparently because of relatively large polar head groups in comparison with their alkyl chains. The size of vesicles depended on the cholesterol content, charge incorporation or hydrophilicity of surfactants. Entrapment of insulin in bilayer structure of niosomes protected it against proteolytic activity of α-chymotrypsin, trypsin and pepsin in vitro. The maximum protection activity was seen in brij 92/cholesterol (7:3 molar ratios) in which only 26.3 ± 3.98% of entrapped insulin was released during 24 h in simulated intestinal fluid (SIF). The kinetic of drug release for most formulations could be best described by Baker and Lonsdale equation indicating diffusion based delivery mechanism. These results indicate that niosomes could be developed as sustained release oral dosage forms for delivery of peptides and proteins such as insulin.

Keywords: Insulin; Niosomes; Oral delivery; Polyoxyethylene alkyl ethers; Proteolytic enzymes

1. Introduction

The delivery of protein pharmaceuticals to the systemic circulation through oral administration is hindered by numerous barriers, including proteolytic enzymes, sharp pH gradients and low epithelial permeability (Lee and Yamamoto, 1990). Recently, different systems and technologies such as enteric-coated capsules (Hosny et al., 2002), gel beads (Sriamornsak, 1998), intestinal patches (Whitehead et al., 2004), liposomes (Takeuchi et al., 1996; Kim et al., 1999; Iwanaga et al., 1999; Kisel et al., 2001; Wu et al., 2004), microparticles (Agarwal et al., 2001; Morcol et al., 2004; Morishita et al., 2004), microspheres (Morishita et al., 1992), mucoadhesive tablets (Caliceti et al., 2004; Krauland et al., 2004), nanocubicles (Chung et al., 2002), nanospheres (Damge et al., 1997; Carino et al., 2000; Foss et al., 2004), and non-ionic surfactant vesicles (Yoshida et al., 1992) have been used to overcome these barriers and improve protein absorption following oral delivery. Among these, vesicular systems such as liposomes have been investigated more than the other systems. Unfortunately, a number of serious limitations exist with the use of liposomes such as in vitro (Ausborn et al., 1992) and in vivo (Poste, 1983) instability.

One alternative of phospholipids, the main constituents of liposomes, is the hydrated mixture of cholesterol and non-ionic surfactants such as alkyl ethers, alkyl esters or alkyl amides non-ionic surfactants (Manosroi et al., 2003). This type of vesicle formed from the above mixtures has been known as niosomes or non-ionic surfactant vesicles (NSVs). The low cost, greater stability, ease of storage and also large numbers of available vesicle forming non-ionic surfactants make these vesicles more attractive than liposomes for industrial production both in pharmaceutical and cosmetic applications (Uchegbu and Vyas, 1998).

Both hydrophilic and hydrophobic substances can be embedded in niosomal vesicles. Some proteins and peptides such as alpha-interferon (Niemiec et al., 1995), bovine serum albumin (Brewer and Alexander, 1992; Murdan et al., 1999),

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cyclosporine A (Niemiec et al., 1995; Waranuch et al., 1998), 9-desglycinamide-8-arginine vasopressin [DGAVP] (Yoshida et al., 1992), GnRH-based anti-fertility immunogen (Ferro et al., 2004), haemagglutinin (Murdan et al., 1999), influenza viral antigens (Chattaraj and Das, 2003), insulin (Khaksa et al., 2000; Varshosaz et al., 2003), luteinizing hormone releasing hormone [LHRH] (Arunothayanun et al., 1999), ovalbumin (Brewer et al., 1998; Rentel et al., 1999) and recombinant human granulocyte-macrophage colony stimulating factor [rhGM-CSF] (Memisoglu et al., 1997) have been successfully encapsulated in niosomes. The encapsulation of pharmaceutical materials in niosomes can decrease drug toxicity, increase drug absorption, stability or activity and retard removal of drug from the circulation due to slow drug release.

In previous article we reported the encapsulation of insulin in sorbitan ester vesicles which led to protection of protein against proteolytic enzymes and sustained release of insulin (Varshosaz et al., 2003). In this study, the ability of polyoxyethylene alkyl ethers (C_nEO_m, Brij^{TM}) mixed with cholesterol to form bilayer vesicles and encapsulate recombinant human insulin was studied. Furthermore, encapsulation efficiency of insulin, protection against enzymes, thermal analysis and size distribution study of vesicles and characterization of niosomes with optical microscope were carried out.

2. Materials and methods

Recombinant human insulin (27.5 IU/mg, Eli Lilly, France) was a kind gift from Exir Pharmaceutical Co., Iran. The non-ionic surfactants used as vesicle-forming materials were Brij 52 (polyoxyethylene 2 cetylether, $C_{16}EO_2$), Brij 72 (polyoxyethylene 2 stearylether, $C_{18}EO_2$), Brij 92 (polyoxyethylene 2 oleylether, $C_{9=9}EO_2$), Brij 76 (polyoxyethylene 10 stearylether, $C_{18}EO_{10}$), Brij 97 (polyoxyethylene 10 oleylether, $C_{9=9}EO_{10}$), Brij 58 (polyoxyethylene 20 cetylether, $C_{16}EO_{20}$) and Brij 35 (polyoxyethylene 23 laurylether, $C_{12}EO_{23}$) and were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Pepsin (from porcine stomach mucosa, 3200–4500 U/mg protein), α-chymotrypsin (from bovine pancreas, 40–60 U/mg protein), trypsin (from bovine pancreas, 10,000 BAEE U/mg protein) and dicetylphosphate (DCP) were also obtained from Sigma. Cholesterol (Chol) was bought from Fluka, Switzerland. Immunoradiometric assay kit was purchased from Biosource, Belgium. All organic solvents and the other chemicals were of analytical grade and were obtained from Merck, Germany.

2.1. Preparation of non-ionic surfactant vesicles

Multilamellar vesicles (MLV) were prepared by modification of hand-shaking method (Baillie et al., 1985) in which 300 μmol of surfactant or surfactant/Chol or surfactant/Chol/DCP mixtures were dissolved in 10 ml chloroform in a 100-ml round-bottomed flask. The organic solvent was then removed at 50 °C, under reduced pressure, in a rotary evaporator (Buchi, Switzerland). The flask was dried overnight in a desiccator to remove any residual solvent. The dried lipid film was hydrated with 5 ml phosphate buffered saline (PBS) (pH 7.4) containing insulin (20 IU/ml) with a gentle rotation in water bath at 55 °C for 10 min. The resulting multilamellar non-ionic surfactant vesicle dispersions were then left to cool slowly. To study the effect of composition of the vesicles, a series of formulations with different molar ratios (m.r.) were designed (Table 1).

Table 1
Vesicle forming ability of studied polyoxyethylene alkyl ethers (Brij^{TM}) non-ionic surfactants; non-ionic surfactant vesicles (NSVs, niosomes) were prepared by classic film method

Surfactant	HLB	Surfactant/cholesterol molar ratio				
		10/0	9/1	7/3	6/4	5/5
Brij 35	16.9	−	−	+[a,b]	+[a,b]	+[a]
Brij 52	5.3	+[c]	+[c]	+[c]	+[c]	+[c]
Brij 58	15.7	−	−	+	+	+[a]
Brij 72	4.9	+	+	+	+	+
Brij 76	12.4	+	−	+	+	+
Brij 92	4.9	+[c]	+[c]	+[c]	+[c]	+[c]
Brij 97	12.4	+	−	+	+	+

[a] Some cholesterol crystals were observed by optical microscopy.
[b] The number of formed niosomes was relatively low.
[c] Creaming was seen after standing of formulation.

2.2. Differential scanning calorimetry analysis (DSC)

A small amount (typically 5 mg) of freeze-dried NSVs (9:1 and 7:3 surfactant/Chol m.r.) or pure semisolid surfactant (Brij 52, Brij 72, and Brij 76) was sealed in a 40-μl-aluminium crucible. For preparation of freeze-dried samples, 1 ml of niosomal suspension was frozen in liquid nitrogen and freeze dried all over the night in a lyophilizer (FD-81, Eyela, Japan). A second crucible containing the equivalent amount of PBS (pH 7.4) was sealed as the reference cell. The temperature of the pans was raised from 20 to 80 °C, at a rate of 5 °C/min and a sensitivity of 1 mW/s using a differential scanning calorimeter (TA 4000, Mettler, Germany). The heat flow calibration was performed with indium. The reproducibility of the thermograms was determined by repeating the temperature cycle three times for each sample.

2.3. Measurements

The particle size and particle size distribution of niosomes were determined by laser-light scattering (Mastersizer X, Malvern Instruments, UK). Some micrographs were prepared by a camera attached to the optical microscope (HFX-DX, Nikon, Japan) in 10 × 40 and 10 × 100 magnifications. Immunoreactive insulin in samples was measured using An INS-IRMA^{TM} radioimmunoassay kit using a gamma counter device (Minigamma Gamma counter, LKB Wallac, Turku, Finland).

2.4. Insulin encapsulation efficiency

To separate non-entrapped insulin, the vesicle suspensions were centrifuged (90 XL Ultracentrifuge, Beckman, USA) at 40,000 × g for 30 min at 4 °C and washed with PBS (pH 7.4). The amount of insulin in the supernatant and also in the