European Journal of Pharmaceutics and Biopharmaceutics 97 (2015) 223–229

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research Paper

Effective incorporation of insulin in mucus permeating self-nanoemulsifying drug delivery systems

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ARTICLE INFO

Article history:
Received 10 October 2014
Revised 15 April 2015
Accepted in revised form 21 April 2015
Available online 29 April 2015

Keywords:
Mucus
SNEDDS
Ionic complexation
Insulin
Oral delivery
Dimyristoyl phosphatidylglycerol

ABSTRACT

The development of a novel, mucus permeating SNEDDS formulation for oral insulin delivery containing a hydrophobic ion pair of insulin/dimyristoyl phosphatidylglycerol (INS/DMPG) is presented. Three oil/surfactant/cosurfactant combinations and 27 weight ratios of oil, surfactant and cosurfactant for each combination were evaluated with the aid of ternary phase diagrams, for the incorporation of the protein/phospholipid complex. The developed formulation was characterized by an average droplet diameter of 30–45 nm. Depending on the initial protein concentration, the loading of insulin in SNEDDS varied between 0.27 and 1.13 wt%. The therapeutic protein was found to be efficiently protected from enzymatic degradation by intestinal enzymes (i.e., trypsin, α-chymotrypsin). The SNEDDS formulation exhibited increased mucus permeability and did not appear to be affected by ionic strength. The incorporation of INS/DMPG in SNEDDS prevented an initial burst release of insulin. INS/DMPG loaded SNEDDS were found to be non-cytotoxic up to a concentration of 2 mg/ml. According to the reported results, the incorporation of the hydrophobic ion pair of INS/DMPG in SNEDDS could be regarded as a promising strategy for the oral delivery of insulin.

1. Introduction

Despite the significant barriers to drug delivery in the gastrointestinal tract (GIT), the oral route continues to be the most intensively studied for the administration of protein- and peptide-based drugs (P/P drugs). The interest in the oral route is well appreciated by considering its obvious advantages (e.g., ease of administration without requiring sophisticated sterile manufacturing facilities and/or the direct involvement of health care professionals, large patient acceptability, etc.) [1]. The barriers to P/P absorption via the gastrointestinal tract (GIT) are primarily chemical, enzymatic, as well as penetration related (e.g., mucus layer, intestinal epithelium). The low oral bioavailability of macromolecules (i.e., <1%) and their short in vivo half lives (<30 min) [2] are due primarily to their large molecular weight (>3000 Da) and their hydrophilicity (i.e., absorption by passive diffusion is limited to lipophilic drugs with MW <700 Da) [2,3]. To overcome these barriers and improve the bioavailability of P/P drugs, various approaches have been examined, including the development of nanocarrier-based drug delivery systems (DDS) capable of traversing the deleterious mucosal environment and maintaining therapeutic drug levels without side effects. To date polymer, lipid and oil based nanocarriers have been employed for the oral delivery of P/P drugs and antigens [4].

Self-nanoemulsifying drug delivery systems (SNEDDS) are isotropic mixtures of oil, surfactant and cosurfactant spontaneously forming an O/W nanoemulsion upon mixing with water. The formed nanoemulsion is a thermodynamically stable system with extremely small droplet size (i.e., ⩽50 nm). Following their oral administration, drug loaded SNEDDS can rapidly disperse in gastrointestinal fluids resulting in the formation of drug containing nanodroplets which could diffuse through the mucus gel layer, as demonstrated by Friedl et al. [5], and be uptaken by epithelial cells. Due to their anhydrous nature, SNEDDS can be also filled directly into soft or hard gelatin capsules in order to facilitate their administration [6–10]. SNEDDS are typically used to enhance the oral bioavailability of poorly water soluble drugs and there already exist marketed formulations of hydrophobic drugs based on SNEDDS technology [11]. On the other hand, the administration of hydrophilic drugs including therapeutic peptides is considered extremely challenging [10]. Venkata Ramana Rao and Shao, loaded

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http://dx.doi.org/10.1016/j.ejpb.2015.04.013
0939-6411/

β-lactamase (BLM) into SNEDDS via a solid dispersion technique [7]. It was shown that, following the oral administration of a BLM loaded SNEDDS formulation to rats the oral bioavailability of BLM was significantly increased (6.34%) compared to BLM solution [12]. Ruan and coworkers [8], illustrated the potential use of phospholipids/drug complexes in the preparation of SNEDDS to enhance the oral bioavailability of water soluble drugs (e.g., matrine). The absolute bioavailability of matrine was shown to drastically increase from 25% to 84.6% due to the SNEDDS formulation, suggesting its great potential for clinical application. Qi et al. [9], developed self-double-emulsifying drug delivery systems (SDEDDS) to improve the oral absorption of pidotimod, a peptide-like drug with high solubility and low permeability. Plasma concentration–time profiles from pharmacokinetic studies in rats dosed with SDEDDS showed a 2.56-fold ($p < 0.05$) increased absorption of pidotimod, compared to its solution. More recently, Zhang and coworkers [13] complexed insulin with soybean phosphatidylcholine (SPC) by dissolving the protein and the phospholipid in an appropriate solvent and subsequently lyophilizing the solution. The formed complex was incorporated into a SNEDDS formulation, which following oral administration to diabetic rats resulted in an increase of the relative bioavailability of insulin up to 7.15% as compared to the 0.11% of the insulin solution. Sakloetsakun et al., [14] incorporated insulin in SNEDDS in the presence of chitosan and/or thiolated chitosan. Thiolated chitosan was found to increase the encapsulation efficiency of protein in SNEDDS. In vivo results showed that serum insulin concentration of insulin/chitosan-TGA SNEDDS displayed a significant difference (p-value = 0.02) compared to oral insulin solution. Hintzen and coworkers [15] developed a self-microemulsifying drug delivery system (SMEDDS) incorporating a hydrophobic complex of the model peptide drug leuprorelin with sodium oleate. An in vivo study in rats showed a 17.2-fold improved oral bioavailability of leuprorelin for the SMEDDS formulation in comparison with a leuprorelin control solution.

Due to its net positive charge at acidic pH, insulin could be associated with anionic amphiphilic molecules (e.g., surfactants, phospholipids, fatty acids) via ionic complexation in order to allow its efficient incorporation in SNEDDS [16]. In this study, a novel mucus permeating SNEDDS formulation for oral insulin delivery, incorporating a hydrophobic ion pair of insulin/dimyristoyl phosphatidylglycerol (INS/DMPG) was developed. Three oil/surfactant/cosurfactant combinations and 27 weight ratios of oil, surfactant and cosurfactant for each combination were initially evaluated with the aid of ternary phase diagrams indicating the self-nanoemulsifying region of each combination. One weight ratio from each combination was selected for the incorporation of the INS/DMPG complex. The selected SNEDDS were examined with respect to mucus permeability, electrolyte tolerance and cytotoxicity. The effect of the initial insulin concentration on the protein loading was also evaluated, as well as the protection of insulin from enzymatic degradation by intestinal enzymes (i.e., trypsin, α-chymotrypsin). Finally, the release of insulin from SNEDDS was experimentally investigated.

2. Materials and methods

2.1. Materials

Porcine insulin (⩾27 USP units/mg), dimyristoyl phosphatidylglycerol (DMPG), trypsin from bovine pancreas (~10,000 BAEE units/mg protein), α-chymotrypsin from bovine pancreas (TLCK treated, ⩾40 units/mg protein), Cremophor EL®, fluorescein diacetate (FDA) and phosphate buffered saline (PBS, 10x, pH 7.4) were purchased from Sigma. Free samples of Lauroglycol FCC®, Labrafil M1944CS® and Transcutol P® were generously provided by Gattefossé. All other reagents were of analytical grade and commercially available. Purified porcine intestinal mucus was kindly provided by the Institute for Cell and Molecular Biosciences, Newcastle University (Newcastle upon Tyne, United Kingdom). Caco-2 cells were kindly provided by the Josef Stefan Institute (Ljubljana, Slovenia).

2.2. Preparation and characterization of SNEDDS

Lauroglycol FCC and Labrafil M1944CS were used as oils, Cremophor EL was used as a surfactant and Transcutol P and Labrafil M1944CS were used as cosurfactants for the preparation of SNEDDS. More specifically, three oil/surfactant/cosurfactant combinations were examined (e.g., Lauroglycol FCC/Cremophor EL/Labrafil M1944CS, Lauroglycol FCC/Cremophor EL/Transcutol P, Labrafil M1944CS/Cremophor EL/Transcutol P [17]) and a 3^3 full factorial design was adopted to select the weight ratios of oil, surfactant and cosurfactant to be evaluated for each combination (Table 1). Mixtures of oil, surfactant and cosurfactant at predefined weight ratios, comprising the oil phase, were sonicated in an ice bath with the aid of a microtip sonicator (*Sonicator Sonics Vibra Cell VC-505*) at a 40% amplitude for 45 s to achieve homogeneous mixing of the ingredients. 100 mL of water was subsequently added dropwise under magnetic stirring to 1 g of the oil phase to form nanoemulsions. Ternary phase diagrams, indicating the nanoemulsion region, were constructed for each oil/surfactant/cosurfactant combination (Fig. 1). The oil/surfactant/cosurfactant weight ratios which resulted in the spontaneous formation of a transparent nanoemulsion constituted the self-nanoemulsifying region in the ternary phase diagram of each combination. One mixture of oil/surfactant/cosurfactant from the self-nanoemulsifying region (i.e., SNEDDS) was selected from each combination for the incorporation of the INS/DMPG complex (see SNEDDSa,b,c in Table 2). SNEDDS labeled with fluorescein diacetate (FDA) were also prepared by dissolving FDA in the mixture of oil/surfactant/cosurfactant at a concentration equal to 0.2 w/w%.

Table 1
3^3 Full factorial experimental design adopted for the selection of the weight ratios of oil, surfactant and cosurfactant to be evaluated for each SNEDDS combination.

Oil mass (mg)	Surfactant mass (mg)	Cosurfactant mass (mg)
3	3	0
		1.5
		3
	5	0
		1.5
		3
	7	0
		1.5
		3
5	3	0
		1.5
		3
	5	0
		1.5
		3
	7	0
		1.5
		3
7	3	0
		1.5
		3
	5	0
		1.5
		3
	7	0
		1.5
		3