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Enhancing bioavailability and controlling the release of glibenclamide from optimized solid lipid nanoparticles

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ABSTRACT

The objective of this study was to explore the possibility of incorporation of a poorly water soluble drug glibenclamide (GLB) into solid lipid nanoparticles (SLNs), which offer advantages of extended drug release and improved oral bioavailability. SLNs were prepared via emulsification followed by ultrasonication technique. Solubility studies were performed to identify appropriate lipid for preparation of SLNs. The effect of inclusion of different types of lipids and surfactants was investigated. Pharmacokinetic, pharmacodynamic and histological finding of optimized SLNs were performed on rats. Stability of GLB-SLNs at 25 °C and 4 °C was investigated. Results revealed that changing type or concentration of lipid or surfactant had a pronounced effect on entrapment efficiencies (E.E), particle size, and release behavior of the SLNs. Physicochemical characterization showed GLB was in amorphous state, nanometer range and pharmacophore was preserved. GLB-SLNs showed extended drug release than dissolution of GLB suspension. Oral administration of optimized SLNs to diabetic rats led to significant reduction in blood glucose level with short onset time (0.5 h) and long duration of effect (24 h). Stability study recommended 4 °C as optimum storage temperature of SLNs. In this study, we confirmed that SLNs of GLB had beneficial effects on controlling diabetes in diabetic rats.

1. Introduction

Diabetes mellitus is an endocrine metabolic disorder associated with elevated blood glucose level. The global prevalence of diabetes between adults over 18 years of age has increased from 4.7% in 1980 to 8.5% in 2014 [1]. Diabetes is a major cause of blindness, kidney failure, heart attacks, stroke and lower limbs amputation [2]. The majority of diabetic patients are found to be of type II diabetes mellitus (non-insulin-dependent). In spite of beneficial effects of oral hypoglycemic drugs, studies found that recommended blood glucose level is achieved by less than 50% of diabetic patients due to poor patient adherence to treatments [3]. Glibenclamide (GLB) is a potent oral hypoglycemic drug belongs to the second generation of sulfonylurea class [4]. GLB used in treatment of type 2 diabetes mellitus through stimulation of β cells of Langerhans in pancreas to release insulin. It may further increase sensitivity of peripheral tissues to insulin along with decreasing hepatic clearance of insulin leading to increase in hormone level [5]. According to Biopharmaceutics Classification System (BCS), GLB can be assigned to BCS class II with low aqueous solubility and high permeability (log $P = 3.79$) that result in low oral bioavailability of GLB (~45%) [6]. GLB has a short elimination half -life of 4 h, which dictate high dose frequency [5]. These specifications resulted in poor adherence to long term treatment with GLB, which can be avoided by development of extended release oral dosage form of GLB. Several approaches have been developed to overcome these problems as solid dispersions [7], amorphization [8], complexation with β cyclodextrin [9], transdermal patch [10], floating tablets [5] and self-nanoemulsifying drug delivery system [6].

Solid lipid nanoparticles (SLNs) are looked as one of the prospective nanotechnology-based drug delivery systems. Their potential advantages such as the possibility of prolonged drug release and targeting, improving dissolution rate and hence bioavailability, lipophilic and hydrophilic drugs can be encapsulated, negligible toxicity, biodegradable and biocompatible, have attracted many researchers exploring the possible use of SLNs as drug delivery systems [11,12]. SLNs are generally composed of solid lipid(s) and surfactant(s). The lipids, main ingredient of SLNs, affect drug entrapment, stability of SLNs and extended release behavior of the

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developed formulations. Different types of lipid can be used such as fatty acids, fatty alcohols, fatty esters and glycerides. Waxes have also been reported for fabrication of SLNs [13]. Surfactants play a significant role in dispersion of melted lipid in the aqueous phase during formulation and obviously stabilize lipid nanoparticles during storage [14,15].

SLNs are generally developed by solvent and non-solvent based techniques. The solvent-based technique employs organic solvent to dissolve the solid lipids forming the liquid emulsion followed by evaporation of the solvent to get SLNs. Non-solvent technique utilizes high energy such as high pressure and shear homogenization to disperse the molten lipid in the aqueous phase [16]. The non-solvent approaches are generally preferred over solvent based ones because of low production cost and lack of organic solvent associated cytotoxicity [15].

Objectives of the present study were to explore the feasibility to encapsulate GLB into SLNs, optimize SLNs for physicochemical properties, and evaluate the pharmacokinetics and pharmacodynamics efficacy in rats. We also studied stability of GLB-SLNs at 4 °C and 25 °C for 3 months.

2. Experimental

2.1. Materials

Glibenclamide (GLB), glyceryl monostearate (GMS), Tween® 80 (polyoxyethylene (20) sorbitan monooleate), alloxan monohydrate, stearic acid and Pluronic® F68 (polyoxyethylene-polyoxypropylene (150:29) block copolymer) were purchased from Sigma Aldrich, Inc. (St.Louis, Missouri). Compritol® 888 ATO (glycerol dibehenate/behenate), cetyl palmitate, Precirol® ATO 5 (glycerol palmitostearate), Gelucire® 43/01 (mixtures of mono, di and triglycerides with PEG esters of fatty acids), Gelucire® 50/13 (stearoyl macrogol-32-glycerides) and Geleol™ mono-and diglycerides (glycerol monostearate 40–55, Type I), were kindly gifted by Gattefosse (St Priest, Cedex, France). Phospholipon® 90 G (soy phosphatidylcholine) was given as a gift from Lipoid (Ludwigshafen, Germany). All the reagents were of analytical grade and used without further purification.

2.2. Solubility studies

Solubility studies of GLB in different lipids (Compritol® 888 ATO, cetyl palmitate, Gelucire® 43/01, Gelucire® 50/13, Precirol® ATO 5, stearic acid, GMS and Geleol™ mono-and diglycerides) were performed to identify suitable lipids for development of the GLB-SLNs. Selections were based on high solubilization extent for GLB, confirming solubilization of GLB in the developed dispersion. The screening of lipids was carried out via test tube method where one gram of lipid was transferred to test tube and the lipid was maintained at temperature above its melting point by 5 °C. The GLB was increased gradually by 1 mg till completely dissolved and amount of drug dissolved in one gram of each lipid was determined [17].

2.3. Formulation of GLB-SLNs

SLNs of GLB were prepared by emulsification followed by ultrasonication technique [14,18]. The selected lipids (GMS, Compritol® 888 ATO, Precirol® ATO 5 or Gelucire® 43/01; 5%), GLB (5% of lipid content) and lipophilic surfactant (Phospholipon® 90 G) in case of using tertiary mixture (1:1:1) were heated to temperature 5–10 °C above the lipid melting point forming the lipid phase. An aqueous phase was prepared by dissolving the hydrophilic surfactant (Tween® 80, Pluronic® F68 or binary mixture of them (1:1)) in distilled water and heated to the same temperature of the lipid phase. Hot aqueous phase was then added to the molten lipid phase and homogenized using homogenizer (WiseMix™ HG15A, Daihan Scientific, Seoul, Korea) at 21,000 rpm for 10 min, this stage produces hot coarse o/w emulsion which was further ultrasonicated using probe sonicator (ultrasonic processor, GE130, probe CV18, USA) for 10 min to produce the nano-emulsion. Nano-emulsion was directly placed in ice box to cool down rapidly and converted into solid nanoparticles dispersed in aqueous phase. An overview of different formulations prepared by changing type of lipids and surfactants was presented in Table 1.

2.4. Physicochemical characterization

The mean particle size, polydispersity index (PDI) and zeta potential of GLB-SLNs were determined using Malvern® Zetasizer Nano ZS90 (Malvern® Instruments Limited, Worcestershire, UK). All measured samples were diluted (1:200) by distilled water. Measurements were performed at 25 °C in triplicate using 90° scattering angle.

Encapsulation efficiency (E.E) of the prepared SLNs was measured by indirect method through measuring the concentration of the free GLB in the aqueous suspension medium [19]. The unencapsulated GLB was measured by centrifugation of 2 ml of SLNs dispersion at 100,000 rpm for 2 h at 4 °C using ultracentrifuge (Beckman Instruments TLX-120 Optima) [20]. The aqueous phase of the dispersion was separated and diluted with 0.05 M borate buffer pH 9.5 and concentration of unencapsulated GLB in the supernatant was measured spectrophotometrically (Shimadzu, the model UV-1800 PC, Kyoto, Japan) at 225.2 nm against blank [21]. Percent of encapsulated GLB into SLNs was calculated using the following equation:

$$\text{Percent of encapsulated GLB} = \frac{\text{Initial amount of GLB in SLNs} - \text{Amount of unencapsulted GLB}}{\text{Initial amount of GLB in SLNs}} \times 100$$

2.5. Dissolution studies

Dissolution tests were performed for pure drug, and selected formulations SLN1, SLN8, SLN12 as well as SLN14 via dialysis bag technique using dialysis membrane with a molecular weight cut-off (MWCO) of 12–14 kDa (Spectrum Laboratories Inc., Rancho Dominguez, CA) [22]. Dialysis membrane was washed before use with distilled water to remove excess glycerin and then soaked overnight in the release medium (0.05 M borate buffer pH 9.5) [21,23]. Five milligrams of pure GLB and equivalent volume of the developed SLNs dispersion were suspended in 2 ml of the release medium into dialysis bag with the two ends fixed by clamps and dipped in the dissolution vessel of USP apparatus II (Erweka TD6R, Germany) containing 500 ml of the release medium at 75 ± 1 rpm and 37 ± 0.5 °C. At predetermined time interval 5 ml was withdrawn from the vessel and replaced with equal volume of the fresh release medium to maintain a sink condition. The samples were