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Solid phospholipid nano-particles: Investigations into formulation and dissolution properties of griseofulvin

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ABSTRACT

Solid phospholipid (PL) nanoparticles with griseofulvin (GRIS) as a model drug were prepared by co-spray drying. Their dissolution properties were compared with formulations containing the physical blends of the native crystalline drug and excipient materials, and physical blends of the spray dried materials. Co-spray drying was performed from ethanol + water solutions (80 + 20) using Büchi Nano Spray Dryer B-90. Dissolution profiles in phosphate buffer (PBS), simulated intestinal fluids (fasted state simulated intestinal fluid (FaSSIF)) and pancreatin containing media (PAN) were studied. It was found that the influence of PL on the dissolution profile was affected by both the solid state of the drug formulation and the dissolution medium: the co-SD formulations showed the fastest release in all media. The amount of GRIS dissolved after 5 h increases by a factor of 7 for the co SD as compared to physical blend of native materials in PBS, and a factor of 4 in FaSSIF respectively. Surprisingly, in contrast to PBS, dissolution rate in FaSSIF decreased with increasing the PL content. All the pancreatin containing media showed a decrease in dissolution rate and extent independently of the processing methods due to an incompatibility between GRIS and PAN.

1. Introduction

Prominent examples of lipid-based oral formulations to tackle the problem of poor aqueous solubility of APIs (active pharmaceutical ingredients) are SEDDS (self emulsifying drug delivery systems) and SMEDDS (self micro emulsion drug delivery systems). These are three- component systems comprising not only the API and lipid, but also considerable amounts of surfactants. The formulations have successfully reached the market (e.g. Cyclosporin-Neoral® and Amprenavir-Agenerase®). Recently, similar formulations were transferred into solid nano-particles (S-NEDDS) (Shanmugam et al., 2011) which may be advantageous in terms of processing, handling and storage stability. The use of phospholipids (PL) appears as an attractive alternative approach in order to produce solid lipid-based formulations devoid of artificial surfactants. In the present study, solid phospholipid nano-particles were produced and studied in the view of oral delivery of poorly soluble drugs.

In addition to the API's physicochemical properties (e.g. solubility, log *P*, etc.), biopharmaceutical characteristics such as total dose, absorption mechanism, and metabolism are important for formulation development. The dissolution behaviour of the API from the formulations in bio-relevant media (Butler and Dressman, 2010; Wu and Benet, 2005; Zaki et al., 2010) is therefore also important for early formulation work.

The dissolution rate may be increased by increasing the surface area of the solid particles (smaller particles), reducing the thickness of the diffusion layer (stirring) or by increasing the solubility of the solid (by alternative particle structure, composition, or dissolution medium).

In this study, attempts to increase the dissolution rate from solid formulations have been undertaken by reducing size and by altering composition and structure of the particles.

Spray drying is a widely used technique to obtain small solid particles. In many cases, a metastable amorphous structure with a higher solubility than the crystalline state is obtained. Spray drying was used in this study to produce solid phospholipid nano-particles (Brinkmann-Trettenes et al., 2014) composed of griseofulvin (GRIS) and excipients.

For the dissolution tests, we refer to (Galia et al., 1998) fasted state (FaSSIF) and fed state (FeSSIF) simulated intestinal fluids. Both media contain phosphate buffer pH 6.5 and sodium taurocholate as well as lecithin. Many drugs are better solubilised due to incorporation into taurocholate micelles and/or into mixed micelles with lecithin. For

Abbreviations: FaSSIF, fasted state simulated intestinal fluid; PL, phospholipid; PC, phosphatidylcholine; GRIS, griseofulvin; TRE, trehalose; SD, spray drying.

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the dissolution of PL containing formulations in FaSSIF the same increase in apparent solubility is expected (Schwebel et al., 2011).

United State Pharmacopoeia (USP) describes simulated intestinal fluid as a solution of 1% pancreatin (i.e. a mixture of porcine pancreas enzymes including phospholipases) in phosphatebuffer pH 6.8 (0.5 M). Pancreatin enzymes cleave one acyl chain from PL, leaving lyso-phospholipids (lyso-PL) and a free fatty acid. The properties of lyso-PL appear particularly attractive for oral drug formulations in order to mimic the physiological conditions upon digestion. Successful approaches have been shown to enable high drug solubilisation by lyso-PL (Van Echteld et al., 1981). In the case of poorly soluble drugs, solubilisation is regarded the first step to enable absorption and thereby to increase bioavailability.

It is our hypothesis that more PL in the formulations would solubilise GRIS better and hence increase both the extent of dissolution as well as dissolution rate. These properties may also depend on the structure of the dry formulations due to different processing. Moreover, we hypothesise that in pancreatin-containing dissolution media the formation of lyso-PLs will further increase dissolution rates due to better solubilisation of the API.

2. Materials and methods

2.1. Materials

Lipoid E80 phospholipid (PL), an egg lecithin containing 80–85% phosphatidylcholine (PC) and 7.0–9.5% phosphatidylethanolamine (manufacturer's specifications) was donated from Lipoid GmbH, Ludwigshafen, Germany. D(+)-trehalose dihydrate (TRE) and griseofulvin (GRIS) of analytical grade were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, USA). Ethanol 96% and acetonitrile (HPLC grade), formic acid, sodiumdihydrogen phosphate monohydrate, sodium hydroxide, sodium chloride of analytical grade and Pancreatin 8×USP standard were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, USA). SIF powder for preparing FaSSIF buffer was purchased from www.biorelevant.com (Surrey, United Kingdom).

2.2. Methods

2.2.1. Spray drying

Spray drying was performed using Büchi B-90 Nano spray dryer (Büchi Labortechnik AG, Flawil, Switzerland) with inert loop B-295 dryer (Büchi Labortechnik AG, Flawil, Swizerland) and N_2 as drying gas. GRIS and/or PLs were dissolved in ethanol. TRE was dissolved in water. The solutions were mixed under magnetic stirring in a fixed ratio of PL:TRE (1 + 1.6 (m + m)) for processing reasons as evaluated in a previous study (Brinkmann-Trettenes et al., 2014). Spray drying was performed from ethanol + water 80 + 20 (m + m) solutions under the following conditions: the speed of the peristaltic pump was set to 12.5 ml/min (pump setting "2"), nozzle size 5.5 μm; air inlet temperature 70 °C and inlet air flow rate 140 L/min with N_2 as drying gas and O_2 concentration at maximum 4%. The temperature of the inert loop was set to 4 °C. During spray drying process, the following parameters were measured: air inlet temperature: 70 °C; air outlet temperature: 42–45 °C; spray head temperature: 72–76 °C; air flow 138–140 L/min.

2.2.2. Preparation of formulations

Nine formulations were studied: three fixed mass combinations of excipients GRIS + PL + TRE (1 + 10 + 16, 1 + 30 + 48, 1 + 100 + 160; m + m + m) were prepared by three production methods: (1) physical blends of the native materials, (2) physical blends of individually spray dried (SD) materials and (3) co-spray dried formulations (co-SD). Physical blends of native material were prepared by carefully triturating native GRIS (crystalline), PL (solid) and TRE (crystalline) using a cooled mortar and pestle. For the individually spray dried formulations (physical blends of SD materials), GRIS and PL/TRE, respectively, were spray dried from ethanolic solutions as described above. These SD materials were blended in a closed container. The co-SD formulations were processed as described above in Section 2.2.1. All samples were stored at room temperature in a desiccator above calcium chloride.

2.2.3. Dissolution and solubility media

Phosphatebuffer (PBS) pH 6.5 was prepared according to Kloefer et al. (2010) by dissolving 0.42 g sodium hydroxide, 3.95 g sodiumdihydrogenphosphate monohydrate and 6.19 g sodium chloride in water to 1 L with adjusting the pH using 1 M sodium hydroxide/hydrochloric acid solutions. FaSSIF buffer was prepared by dissolving 2.24 g SIF powder in 1 L of PBS buffer pH 6.5. $FaSSIF_{PAN}$ and PBS_{PAN} (3.2 mg/ml) was prepared by dissolving Pancreatin 8×USP standard in FaSSIF buffer or PBS buffer pH 6.5 respectively in accordance with Liu et al. (2012).

2.2.4. Dissolution studies

Dissolution studies were performed in pharmacopoeia dissolution apparatus 1 (European Pharmacopoeia, 7th ed.) (Sotax AT 7 smart, Sotax, Allschwil/Basel, Switzerland) in 500 ml of the respective dissolution media at 37 °C with 50 rpm. Circular paper filters were put in the bottoms of the baskets before the respective formulations were weighed in. The total amount of GRIS was equal in each experiment (1.95 mg). Samples were withdrawn at suitable time intervals through tubes permanently placed in the dissolution vessels which were equipped with 0.45 μm filters (Acrodisc 25 mm syringe 0.45 μm filters with Supor® membrane (Pall Corporation, Port Washington, USA)). Sample volumes were replaced by fresh PBS buffer or FaSSIF buffer pH 6.5 respectively, in order to keep the total volume constant.

2.2.5. Quantification of griseofulvin

GRIS was quantified according to the officinal monograph (European Pharmacopoeia, 7th ed.) with the following modifications: a Waters HPLC system (Waters Corporation, Milford, USA) with Waters 2795 separation module, Waters 2489 UV/Visible detector, Empower software (Empower 3 software, Waters Corporation, Milford, USA) and XTerra® RP C18, 5 μm column with dimensions 3.9 × 150 mm (Waters, Dublin, Ireland) or equivalent were used for quantifying GRIS. Mobile phase: 0.1% formic acid in acetonitrile: 0.1% formic acid in water (45:55), column temperature: 35 °C, wavelength: 291 nm. Flow 1 ml/min, injection volume: 20 μl. A standard curve with 7 points and 3 replicates ($r^2 = 0.9947$) was prepared. The retention time of the GRIS peak was approximately 4 min.

2.2.6. Solubility

The thermodynamic solubilities of GRIS in water, PBS buffer pH 6.5, and in TRE solutions (1% and 10%) respectively as well as the apparent solubilities of GRIS, physical blends and SD formulations of GRIS in the respective media (FaSSIF buffer pH 6.5, PBS_{PAN}, and in $FaSSIF_{PAN}$) were determined by the shake flask method. Excess of GRIS, physical blend, or formulation respectively was weighed into a 15 ml falcon tube and filled with 10 ml of the respective medium. The samples were placed in a shaking water bath at 25 °C and the concentration of GRIS was determined after 5–7 days of equilibration by centrifuging samples at 11,000 rpm for 70 min, and filtering the samples through a 0.22 μm filter (Millex®-GV