Colloids and Surfaces B: Biointerfaces 135 (2015) 756–764

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Preparation and pharmacodynamic assessment of ezetimibe nanocrystals: Effect of P-gp inhibitory stabilizer on particle size and oral absorption

Kale Mohana Raghava Srivalli, Brahmeshwar Mishra *

Department of Pharmaceutics, Indian Institute of Technology (Banaras Hindu University), Varanasi, Uttar Pradesh 221005, India

ARTICLE INFO

Article history:
Received 14 February 2015
Received in revised form 16 August 2015
Accepted 24 August 2015
Available online 28 August 2015

Keywords:
Ezetimibe
Nanocrystals
AA2G
TPGS
Pharmacodynamics

ABSTRACT

Drug nanocrystals have been widely accepted as potent formulations to overcome poor solubility, dissolution and bioavailability problems of hydrophobic drugs. The present study aimed to develop drug nanocrystals of ezetimibe (Eze), a model BCS class II and hypocholesterolemic drug using bottom up precipitation methods. D-α-Tocopheryl polyethylene glycol 1000 succinate (TPGS), and L-ascorbic acid-2-glucoside (AA2G), were the two stabilizers whose potential in developing Eze nanocrystals was investigated. Particle size and zeta potential portrayed the potential of both the stabilizers in producing Eze nanocrystals. The optimized nanocrystal formulations were evaluated for *in-vitro* solubility, dissolution, solid state characters and *in-vivo* pharmacodynamic performance. The nanocrystal formulations remarkably increased the solubility of the drug ($p < 0.05$ compared to pure drug). Pure drug could not dissolve more than 28.9% during the 60 min dissolution study whereas the drug nanocrystals prepared with AA2G and TPGS presented $t_{90\%}$ at 41.33 ± 2.58 and 16.07 ± 2.32 min, respectively. The PXRD and DSC studies confirmed the retention of crystallinity and the SEM images indicated lack of aggregation in dried nanocrystals. The TPGS nanocrystals presented significantly superior pharmacodynamic activity upon oral administration. The current study corroborated TPGS nanocrystals to be a promising choice of formulation for the oral delivery of Eze.

1. Introduction

Generation of active pharmaceutical ingredients (APIs) by novel drug discovery technologies like high throughput screening and combinatorial chemistry has been producing clinically proven therapeutically active APIs [1]. However, 70–90% of these clinically proven APIs were identified with low aqueous solubility which is a major hindrance to their oral bioavailability [2]. Oral route is the most preferred route of administration and poor solubility is one of the main challenges to develop oral dosage forms with acceptable bioavailability.

The potential benefit of drug nanocrystals (NCs) in improving the solubility, dissolution and oral bioavailability of poorly soluble drugs has been well established over the last two decades [3,4]. NCs are encapsulating-carrier free nanoparticles and are known for their manufacturing simplicity. NCs are produced either by top down fragmentation or bottom up amalgamation [5]. The USFDA considers an NC product as "new drug" because, its markedly superior and unique pharmacokinetic profile is not bioequivalent or comparable to any other solubilized form of the same drug, not even to the drug's own micronized form, administered at the same dosage [6]. In this study, an attempt was made to prepare NCs of a poorly water soluble drug, ezetimibe (Eze), by bottom up precipitation methods and to study the effect of optimized NC formulations on the pharmacodynamic performance of Eze.

Eze is a model BCS class II drug and a hypocholesterolemic agent. The oral absorption of Eze shows inter-subject variability and its bioavailability could be as low as 35% due to its poor solubility and P-gp efflux [7]. Eze acts by inhibiting the small intestinal absorption of cholesterol [7]. The P-gp molecules at the intestinal brush border cause P-gp efflux of Eze and thus interfere with the absorption of Eze. So far, though few cyclodextrin complexes [8] and cocrystal formulations [9] were attempted, it was only the colloidal drug delivery systems (CDDS) that reported improvement in *in-vitro* dissolution as well as *in-vivo* bioavailability of Eze which signified the effect of nanosize on the improved performance of Eze. Among the different CDDS, Eze was formulated as self nanoemulsifying systems reported in liquid [7] and solid forms [10] and as

* Corresponding author. Fax: +91 542 2368428.
E-mail address: bmishrabhu@rediffmail.com (B. Mishra).

http://dx.doi.org/10.1016/j.colsurfb.2015.08.042
0927-7765/

nanoemulsion formulations [11,12]. All these formulations contained several components which made the optimization of their preparation laborious and time taking. Furthermore, their preparation involved use of large amounts of surfactants and cosurfactants, which, from the toxicological stand point, is a legitimate concern. NCs are one of the CDDS that reached the market fastest as their formulation involves simple dispersion of drug in either aqueous or nonaqueous media containing one or more GRAS stabilizers (drug:stabilizer ratio considered between 2:1 and 20:1 on a weight basis). NCs exist at the epicenter of the CDDS because of their formulation simplicity, lack of drug loading problems, upscalability and ability to reduce bioavailability variations [13]. Currently, there are six licensed and regulatory approved NC products in the market, five of them are oral dosage forms, of which, for four of the products, the rationale for the development of NC based dosage form was specifically, enhanced bioavailability with reduced variations or food effects [14]. Therefore, we aimed to improve the oral absorption of Eze by formulating NCs. Till date, only Gulsun et al. prepared and conducted *in-vitro* characterization of Eze NCs, but, failed to reduce the particle size (PS) effectively. Eze NCs were prepared using Pluronic F 127 as stabilizer and employing ball milling (PS result was 1259 ± 62 nm) and ultrasonic probe (1736 ± 88 nm) methods [15]. In the present study, we investigated the effect of two stabilizers, L-ascorbic acid-2-glucoside (AA2G) and D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) on the NC formation of Eze.

AA2G is a novel hydrophilic (non-surfactant) excipient that has been approved as a food additive and is expected to be used as a principle solubilizer in fat-soluble vitamin formulations and in other cosmetic products [16]. Inoue et al. reported formation of nanoparticles and improvement in aqueous solubility and dissolution properties of clarithromycin on co-grinding with AA2G [16]. To the best of our knowledge, AA2G has not been studied as an NC stabilizer so far. TPGS is a non-ionic surfactant that has been approved as a safe excipient by USFDA and is being widely explored as stabilizer for NCs. It lacks pharmacokinetic interactions with drugs and has a safety record in biomedical applications with no reports on its unrelated pharmacological effects [17]. It has P-gp inhibitory activity, has been widely in use to evade the P-gp efflux of substrate drugs [18,19] and so, we hypothesized that it may aid in improving the absorption of Eze. The objective of the current investigation was to prepare AA2G NCs (ANCs) and TPGS NCs (TNCs) of Eze. The ANCs were prepared by solvent-antisolvent precipitation followed by high speed homogenization and sonication. TNCs were prepared by evaporative precipitation into aqueous solution (EPAS) method with subsequent sonication. Dried NCs obtained by freeze drying were evaluated for solubility, solid state characteristics, dissolution and pharmacodynamic performance.

2. Materials and methods

2.1. Materials

Eze was a kind gift from Lupin Ltd. (Pune, India). TPGS and AA2G were obtained as generous gift samples from Antares Health Products, Inc. (Illinois, USA) and Nagase Pvt., Ltd. (Mumbai, India), respectively. Acetone, mannitol and sodium lauryl sulphate (SLS) were purchased from Merck specialities Pvt., Ltd.

2.2. Preparation of AA2G NCs (ANCs)

NCs were prepared by using 1% w/v Eze and varying AA2G as 0.25%, 0.5% and 1% w/v. Eze and AA2G were dissolved in acetone and distilled water, respectively. 1 mL of 100 mg drug containing acetone solution was added drop wise to AA2G dissolved aqueous phase at 25 °C under continuous magnetic stirring at 1000 rpm. The suspension was stirred for 30 min and homogenized at 15,000 rpm for 20 min. Following homogenization, the suspension was centrifuged and the supernatant was replaced by aqueous AA2G solution (% w/v AA2G corresponding to the formulation batch under study). The suspension was immediately subjected to 5 min intermittent sonication until the drug particles were completely dispersed in AA2G solution. The obtained nanosuspension was freeze-dried to obtain ANCs. The freeze-drying procedure was as follows: 1% w/v mannitol was added as cryoprotectant and the suspension was rapidly frozen at −20 °C for 12 h. Primary freeze drying of the pre-frozen samples was carried out for 24 h at −60 °C and below 15 Pa followed by secondary drying cycle at 20 °C for 8 h.

2.3. Preparation of TPGS NCs (TNCs)

TNCs were prepared by EPAS technique using 1% w/v Eze and varying TPGS concentration as 0.25%, 0.5% and 1% w/v. Eze and TPGS were dissolved in acetone and distilled water, respectively. 1 mL organic phase containing 100 mg drug was added drop wise to aqueous phase at 25 °C under continuous magnetic stirring at 1000 rpm. The suspension was stirred for 30 min at 1000 rpm and then stirred for 3 h at 200 rpm to allow complete evaporation of the organic solvent. Thus obtained suspension was finally subjected to intermittent probe sonication for 5 min and freeze-dried to collect TNCs. The freeze-drying procedure was the same as described for ANCs.

2.4. Preparation of electrostatically stabilized TNCs (ESTNCs)

ESTNCs were prepared in the same way as TNCs with 1% w/v TPGS concentration after including SLS as ionic surfactant in the aqueous phase. The effect of SLS was studied at concentrations, 0.05%, 0.1% and 0.15% w/v.

2.5. Particle size (PS), polydispersity index (PDI) and zeta potential (ZP) measurements

Fixed angle (165°) dynamic light scattering (Delsa Nano C, Beckman coulter) was employed to measure the PS and PDI. The ZP measurements were made by estimating the electrophoretic mobility of particles under applied electric field. Samples were appropriately diluted with distilled water and the measurements were made in triplicate.

2.6. Solid state characterization

Fourier transform infra-red (FTIR) spectra were recorded using FTIR spectrophotometer (FTIR-8400S, Shimadzu Co., Kyoto, Japan) over the range of 4000–400 cm^{-1}. All the samples were co-ground with anhydrous KBr, pelletized and scanned at 20 °C with the number of reference scans set as 20 and a resolution of 4 cm^{-1}. Differential scanning calorimetry (DSC) analyses were carried out using DSC-822^e (Mettler Toledo, AG, Analytical, Switzerland) by heating the samples between 10 °C and 300 °C. DSC of TPGS was obtained by heating from 10 °C to 160 °C. 4–7 mg samples were accurately weighed in aluminium pans and heated at the rate of 10 °C/min under constant nitrogen purging at 10 mL/min to obtain the thermograms. Powder X-ray diffraction (PXRD) patterns were recorded by employing X-ray diffractometer (PW3050/60 X'pert PRO, PANalytical, Netherlands) with Cu anode at 40 kV and 30 mA. Sample holders were filled at 20 °C and PXRD data were collected in the range $10° < 2\theta < 80°$. The surface morphological properties were investigated by SEM (FEI, QUANTA-200, Netherlands). The dry samples were sprinkled gently at 20 °C lab conditions using spatula