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Research Paper

Formulation development of SYN-004 (ribaxamase) oral solid dosage form, a β-lactamase to prevent intravenous antibiotic-associated dysbiosis of the colon

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ABSTRACT

SYN-004 (ribaxamase) delayed release drug product is a multi-particulate, hard capsule for oral delivery of a recombinant β-lactamase enzyme designed to degrade β-lactam antibiotics administered intravenously, and thus prevent colon dysbiosis. Here we describe the development of the SYN-004 enteric coated pellet formulation, which has been tested in multiple clinical trials. Since the SYN-004 drug substance is a buffered liquid, several binder excipients in different ratios were tested to facilitate binding of SYN-004 to sugar spheres. The binding systems were evaluated by droplet pre-evaluation and film casting tests. The most promising formulations were produced in small scale fluidized bed application runs and analyzed by dissolution tests and complementary analytical assays. Hydroxypropyl cellulose was selected as the preferred SYN-004 binding excipient. The formulation included a second, outer coat containing the enteric EUDRAGIT® L 30 D-55 polymer-based formulation to achieve gastric protection, and rapid SYN-004 release in the intestinal tract, when the pH rises above 5.5. Additional formulation improvements resulted in an increase in the SYN-004 load compared to a predecessor oral enzyme formulation (Ipsat P1A). Thus, a novel formulation and process for an orally administered enzyme was developed and used to manufacture drug product for clinical trials.

1. Introduction

There is a well-established association between the use of antibiotics, in particular those belonging to the β-lactam class such as ceftriaxone, and colon dysbiosis (a disruption of the balance of microorganisms of the colon) (Bartlett, 2002). Of particular concern is the development of *Clostridium difficile*-associated diarrhea (CDAD) and antibiotic-associated diarrhea (AAD) (Bartlett, 2002; Carroll and Bartlett, 2011; Drekonja et al., 2011; Larcombe et al., 2016; Zycinska et al., 2016). Thus, there is a need for new medical interventions that reduce the risk of developing these secondary infections that are associated with the use of intravenously (IV) administered β-lactam antibiotics, of which a substantial portion are excreted into the intestine following IV administration (Maudgal et al., 1982; Karachalios and Charalabopoulos, 2002).

SYN-004 (ribaxamase) is a recombinant β-lactamase, formulated as an oral delayed release capsule designed to be administered to patients receiving SYN-004 susceptible IV β-lactam antibiotics. Upon release of SYN-004 drug substance (DS) from the drug product (DP) into the proximal small intestine, SYN-004 degrades susceptible IV administered β-lactam antibiotics that are excreted into the gastrointestinal (GI) tract. It is believed that SYN-004 will prevent undesirable effects including AAD, CDAD, and the development of antibiotic resistant organisms and other secondary infections in the gut related to antibiotic administration. SYN-004 has been tested in multiple clinical trials (Roberts et al., 2016; Kokai-Kun et al., 2017), and is not systemically available when delivered orally in the delayed release formulation (Kokai-Kun et al., 2016). The mechanism of action of SYN-004 is the inactivation of susceptible β-lactam antibiotics by hydrolysis of the amide bond of the β-lactam ring (Deshpande et al., 2004). The active SYN-004 enzyme is manufactured by recombinant DNA technology by fermentation using *E. coli* [reviewed by Tripathi, 2016], and is purified

Abbreviations: AAD, antibiotic-associated diarrhea; CDAD, *Clostridium difficile* associated diarrhea; DP, drug product; DS, drug substance; GMS, glycerol monostearate; HPC, hydroxypropyl cellulose; HPMC, hydroxypropyl methylcellulose; IIG, inactive ingredient guide; PS80, polysorbate-80; RSD, relative standard deviation; SEM, scanning electron microscopy; TEC, triethyl citrate

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to near homogeneity by a single column chromatography step. The final bulk DS is formulated in potassium phosphate buffer at high protein concentration.

A predecessor oral enzyme product, called Ipsat P1A, was formulated as a multi-particulate pellet containing capsule and tested in multiple clinical trials, where it was shown to be safe and well tolerated. In healthy volunteers, oral P1A prevented IV ampicillin-induced alterations in intestinal microflora and the emergence of antibiotic resistance (Tarkkanen et al., 2009). The goal for the P1A drug delivery strategy was to achieve continuous entry of small intact enteric coated sugar pellets into the duodenum through the pylorus. The previous P1A formulation achieved only approximately 6% loading of P1A and used EUDRAGIT® L 30 D-55 as both the binding excipient and as the enteric coat (Tarkkanen et al., 2009). A similar multi-particulate delivery strategy was maintained for the current SYN-004 DP formulation, although several improvements to the formulation were desired, including: 1) reducing the amount of EUDRAGIT® L 30 D-55 by replacing the binding excipient with a common alternative binder, 2) increasing the level of SYN-004 enzyme in the final coated pellet to greater than 10% SYN-004, 3) ensuring a coated pellet formulation with enteric protection of the SYN-004 enzyme during two (2) hours in 0.1 hydrochloric acid followed by rapid release of the enzyme in pH 6.8 buffer, and 4) ensuring a smooth, crack-free surface with low sticking properties. A higher drug loading on the pellets was desired to facilitate a higher dose of active enzyme and potentially smaller capsules. Further, it was desirable to replace the EUDRAGIT® L 30 D-55 binding excipient with an alternative binding excipient, to keep the amount of EUDRAGIT® L 30 D-55 exposure in the intestine below levels published in the Inactive Ingredient Guide (IIG) Database (Inactive Ingredients database, 2017) [http://www.fda.gov/Drugs/InformationOnDrugs/ucm113978.htm]. This article describes the development of the improved SYN-004 DP formulation used in nonclinical and early phase clinical trials.

2. Materials and methods

The purified SYN-004 bulk enzyme used in these studies was provided by a contract manufacturing partner as a liquid of approximately 100 mg/mL protein in a solution of 50 mM potassium phosphate pH 7.2. The excipients and other materials were purchased commercially as follows: sucrose sugar spheres (Pharm-a-spheres neutral 600–710 μm from Hanns G. Werner GmbH & Co. KG, Tornesch, Germany), polysorbate-80 (Merck KGaA, Darmstadt, Germany), glycerin (Merck KGaA, Darmstadt, Germany), lactose (Pharmatose® 450 M, DFE Pharma, Goch, Germany), polyvinyl pyrrolidone (PVP) (Kollidon® K12 and K25, BASF, Ludwigshafen, Germany), hydroxypropyl cellulose (HPC) (Klucel™ EF Pharm, Ashland Specialty Ingredients G.P., USA), Triethyl Citrate (TEC) (CITROFOL® AI, Merck KGaA, Darmstadt, Germany), low viscosity hydroxypropyl methylcellulose (HPMC) (Sheffield Biosciences, USA), Coni-Snap® hard gelatin capsules size #0 white/white (CAPSUGEL Colmar, France), methacrylic acid – ethyl acrylate copolymer dispersion 30% (EUDRAGIT® NM 30 D, Evonik Nutrition & Care GmbH, Darmstadt, Germany), methacrylic acid – ethyl acrylate copolymer (1:1) dispersion 30% (EUDRAGIT® L 30 D-55, Evonik Nutrition & Care GmbH, Darmstadt, Germany), glycerol monostearate 40–55% (GMS) (Imwitor® 900K, Sasol, Witten, Germany). The CENTA substrate used to determine SYN-004 β-lactamase activity was from Merck Calbiochem (CAS 9073-60-3). Other laboratory equipment used, such as stirrers, mixers, and balances were standard equipment, and manufacturing equipment used are described as presented below.

2.1. Binding excipient screening by casting of free films

Alternative binding excipients to replace EUDRAGIT® L 30 D-55 as the API binding agent were screened by droplet pre-evaluation and film application testing. First, a suspension of SYN-004 was mixed with various binder excipients and at various ratios [Polyvinylpyrrolidone (PVP) K-12, PVP K-25, Hydroxypropylcellulose (HPC), Hydroxypropyl methylcellulose (HPMC)]. Next, a film application plate (Erichsen Coatmaster 500, Erichsen GmbH, Germany) pre-heated to 35–40 °C was covered with aluminum foil. Approximately 0.5 mL of each solution was added to the foil as droplets, then a film was created by drying the solution for 10–24 h at room temperature (20–25 °C). Once dried, the film was qualitatively evaluated by macroscopic optical visualization. Furthermore, the dried casted films were twisted slightly in order to evaluate the mechanical stability. This test was performed to rate the film properties on a matrix, which was used as a first indication to support the selection of a suitable binder excipient and binder:API ratio. Therefore, in some cases, a plasticizer, a glidant, or other common suitable excipient was added to increase the flexibility and stability of the film.

2.2. Fluid bed coating of sucrose sugar spheres

The manufacture of SYN-004 delayed-release capsule was a three stage sequential process comprised of: 1) SYN-004 DS layering onto sugar spheres by spray application, 2) enteric coating with EUDRAGIT® L 30 D-55 by spray application, and 3) encapsulation of pellets into size 0 hard capsules. Encapsulation for analytical testing was manual; encapsulation for the clinical batch was conducted on a Labby capsule filler (MG2, Italy). During the formulation development, two different scales of fluid bed equipment were used for each of the spray application steps. The early drug layering and enteric coating trials were conducted using the Oystar Huttlin Mycrolab Fluid Bed Dryer (Huttlin GmbH, Germany) with bottom spray and microclimate technology (up to 80 g starting spheres). The spray gun used on the Oystar Huttlin equipment was a Huttlin 3-component nozzle with a 0.6 mm nozzle bore. Larger scale batches were produced using the GPCG-3.1 with bottom spray (Glatt GmbH, Germany) (up to 930 g starting spheres). The spray gun used on the GPCG-3.1 equipment was a Schlick 970/0-S3 (Dusen-Schlick GmbH, Germany) with a 0.8 mm nozzle bore for the drug layering and 1.2 mm nozzle bore for the enteric polymer coating.

2.3. Pellet morphology analysis

Scanning Electron Microscopy (SEM) was performed to study the cross-sectional and surface area morphology of the polymeric films on coated sugar spheres. As a preparation step, the samples were broken and sputter-coated with gold for conductance. Examination of the samples was carried out using a Jeol JSM-840 A instrument (Jeol GmbH, Germany) operating at an accelerating voltage of 5 kV.

2.4. Analytical methodology

Dissolution testing based on the United States Pharmacopeia (USP) methodology (USP<711> for Apparatus 2) for delayed release dosage forms was employed. The method used dissolution conditions of 2 h in 0.1N HCl pH 1.2, followed by 4 h in phosphate buffer pH 6.8. Samples were collected after 2 h in acid, then at 15, 30, 45, 60, 120, and 240 min at pH 6.8. The samples were tested for β-lactamase activity using the CENTA substrate (Jones et al., 1982) in a microtiter plate based assay. The assay involves pipetting 50 μL of standards (0.05–0.65 mg/L range), control, blank, or dilutions of unknown samples into microtiter plate wells, then equilibrating the plate for 20 min at 25 °C. Then 200 μL of CENTA solution (1 mM working solution, also equilibrated for 20 min at 25 °C) is pipetted into wells A1-A8 using a multi-channel pipet, and the row is read immediately using a plate reader with wavelength set at 405 nm, measuring every 8 s to collect 8 data points. Additional samples were similarly analyzed in turn, in rows B, C, and so on. The concentrations of unknown samples were interpolated from the standard curve, and the results were plotted as β-lactamase activity versus sampling time of the dissolution analysis.