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Cardiovascular safety pharmacology studies in dogs enabled for a poorly soluble molecule using spray-dried dispersion: Impact on lead selection

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ABSTRACT

The aim of this study was to identify an adequate formulation for a poorly soluble lead molecule (BI-A) that would achieve sufficiently high plasma concentrations after oral administration in dogs to enable a robust cardiovascular safety pharmacology assessment in telemetry-instrumented conscious dogs during lead optimization in drug discovery.

A spray-dried dispersion of BI-A (BI-A-SDD) containing a 1:2 ratio of BI-A and hydroxypropyl methylcellulose acetate succinate-LF was prepared using a Büchi spray dryer B-90 (B-90). Physical form characterization, an in vitro dissolution test and a preliminary pharmacokinetic (PK) study following oral administration of BI-A-SDD were performed. Thereafter, effects on cardiovascular parameters in conscious, chronically-instrumented dogs were investigated for 24 h after a single oral dose (5, 10, and 50 mg/kg) using a modified Latin square cross-over study design.

The BI-A-SDD powder was confirmed to be amorphous and was stable as an aqueous suspension for at least 4 h. The BI-A-SDD suspension provided a greater rate and extent of dissolution than the crystalline BI-A suspension and the supersaturation was maintained for at least 4 h. In PK studies the C_{max} of the BI-A-SDD formulation (25.4 μM; 77-fold the projected efficacious C_{max} of 0.33 μM) was 7.5-fold higher than the C_{max} observed using oral administration of a 10% hydroxypropyl-β-cyclodextrin formulation at 100 mg/kg in dogs (3.4 μM). In conscious, chronically-instrumented dogs, the doses tested and plasma concentrations achieved were sufficient to enable a robust safety pharmacology evaluation. Multiple off-target hemodynamic effects were detected including acute elevations in aortic blood pressure (up to 22% elevation in systolic and diastolic blood pressure) and tachycardia (68% elevation in heart rate), results that were confirmed in other in vivo models. These results led to a deprioritization of BI-A.

The study demonstrated that a spray-dried dispersion, prepared using the B-90 in drug discovery, enhanced the oral exposure of a poorly water-soluble molecule, BI-A, and thereby enabled its evaluation in safety pharmacology studies that ultimately resulted in deprioritization of BI-A from a pool of lead compounds.

1. Introduction

During lead optimization in drug discovery, safety pharmacology studies are routinely integrated into the process for selecting a candidate for preclinical development (U.S. Department of Health, Human Services, Food and Drug Administration, 2001). Safety pharmacology studies, including those performed in higher-species (e.g. dog, nonhuman primate) for cardiovascular safety assessment, are typically performed to assess potential effects on organ function at therapeutic-to-supratherapeutic plasma concentrations (Morimoto et al., 2015), typically upwards of 30-fold above the projected clinically-efficacious C_{max}. Inadequate plasma exposure due to poor physicochemical properties (i.e., poor

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solubility) can result in safety assessments performed only at low multiples of the therapeutic concentration.

While a simple aqueous solution or suspension formulation is preferred for in vivo safety pharmacology studies, it is not uncommon that compounds require enabling formulations to be adequately absorbed due to insufficient solubility. However, the excessive use of solubilizing excipients, such as surfactants, co-solvents, lipids, or complexing agents, can result in undesirable physiological and/or toxicological effects in vivo (Pestel et al., 2006).

In an effort to conduct cardiovascular safety studies in conscious dogs with a Boehringer Ingelheim Pharmaceuticals investigational compound (BI-A), common formulation approaches using aqueous media, co-solvents, and hydroxypropyl-β-cyclodextrin (HP-β-CD) were initially evaluated. Owing to the limited solubility of BI-A in all tested vehicles, the HP-β-CD formulation was tested in dogs but resulted in insufficient plasma concentrations following oral administration (3 – 7-fold the predicted therapeutic C_{max}). To address this challenge, the amorphous solid dispersion (ASD) approach was used to improve the exposure of BI-A in dogs. The ASD consists of an amorphous drug substance stabilized by polymer carriers to produce a system with improved solubility, enhanced dissolution rate, and the ability to sustain high drug concentrations in gastrointestinal medium for improved absorption (Leuner and Dressman, 2000; Newman et al., 2012; Padden et al., 2010). Although the application of the ASD approach to enhance bioavailability of poorly soluble compounds has been increasingly used at late stages of the drug development process, this approach has had very limited applications in drug discovery (Li and Zhao, 2007; Maas et al., 2007; Shah et al., 2014). It is primarily because preparing reliable ASDs at the milligram scale is technically challenging, and therefore, would require a considerable amount of drug substance and development time. We recently reported that the vibrating mesh spray dryer B-90 (B-90) is an effective approach to produce amorphous spray-dried dispersions (SDDs) with greater than 85% yields using small quantities of compounds (Gu et al., 2015). We have also reported that hydroxypropyl methylcellulose acetate succinate-LF (HPMC-AS) as a polymer carrier was well tolerated in vivo when tested at high doses (up to 300 mg/kg) across a battery of pre-clinical safety pharmacology models including those assessing effects on CNS, gastrointestinal and cardiovascular function *(Fryer et al., submitted to Frontiers in Pharmacology)*. In the present study, we utilized the B-90 technology and HPMC-AS as the polymer carrier at the stage of drug discovery, and the ultimate aim of this study was to prepare BI-A as an SDD (BI-A-SDD) formulation to enhance plasma drug concentrations in dogs after oral administration and enable a robust safety pharmacology cardiovascular assessment during lead optimization.

2. Materials and methods

2.1. Materials

BI-A was synthesized by Boehringer Ingelheim Pharmaceuticals (Ridgefield, CT). Crystalline BI-A was used for formulation and SDD preparations and solubility measurements. HPMC-AS was purchased from Shin-Etsu Chemical Co. Ltd. (Tokyo, Japan). 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD) was purchased from Cargill, Inc. (Hammond, IN). All other chemical reagents used in this study were of ACS-grade purity.

2.2. Solubility measurement

The equilibrium solubility of BI-A was determined in pH 2.2, 4.5 and 6.8 aqueous buffers, fasted-/fed-state simulated intestinal fluids (FaSSIF/FeSSIF), neat organic solvents and oils (Labrasol®, Labrafil M 1944®, Capmul MCM®, Miglyol 812N®, and Capryol 90®, propylene glycol, polyethylene glycol), and 10% HP-β-CD. After the bulk drug was stirred in the vehicles for 24 h at room temperature, the undissolved drug residues were filtered through a 0.45 μm pore-size filter and the filtrate was diluted and analyzed by high-performance liquid chromatography (HPLC). HPLC analyses of samples were performed on an Agilent 1100 series system using reversed-phase C18 column (Agilent Zorbax, 1.8 μm, 4.6 × 50 mm) with a mobile phase consisting of 0.1% trifluoroacetic acid in water (A)/0.1% trifluoroacetic acid in acetonitrile (B), gradient of A (%) = 90, 10, 10, 90 at 0, 2.5, 5 and 5.1 min, respectively, and a total run time of 7 min. The flow rate was maintained at 1 ml/min, and samples were analyzed using an online diode array detector at 296 nm. Samples were prepared in acetonitrile/water (50/50, v/v), and a standard curve for BI-A was generated for quantitation.

2.3. Preparation of spray-dried dispersions (SDDs)

BI-A-SDD powders were prepared by means of spray drying using B-90 (BÜCHI Labortechnik AG, Flawil, Switzerland). BI-A and HPMC-AS in a 1:2 (w/w) ratio were dissolved in acetone to produce a 1.5% (w/v) solution for the spray-drying process. The polymer solution was prepared by dissolving the polymer in acetone first and centrifuged at 3000 rpm for 15 min followed by dissolving the study drug in the supernatant to avoid potential nozzle blockage. The resulting solution was then used for spray drying. For the spray-drying process, the flow rate, air flow rate and inlet temperature were set at 2, 120 l/min, and 75 °C, respectively, and a 7-mm mesh nozzle cap was used. Dried powders were collected from the particle collecting chamber using a scraper, and the collected powders were further dried in a vacuum oven at 40 °C overnight.

2.4. X-ray powder diffraction (XRPD)

XRPD patterns of samples were obtained by a MiniFlex II powder X-ray diffractometer (Rigaku Corp., Japan). The X-ray was applied at a voltage of 30 kV and a current intensity of 10 mA. The samples were analyzed over a 2θ range of 3°–35° with a scanning rate of 0.5°/min.

2.5. Differential scanning calorimetry (DSC)

The glass transition temperature (Tg) of samples was evaluated using a TA Q1000 modulated differential scanning calorimeter (TA Instruments, New Castle, Delaware). Sample amounts of 5–8 mg were loaded in T-zero pans and scanned at a rate of 3 °C/min from

Table 1
Solubility of BI-A in aqueous buffers, biorelevant media and organic solvents.

Media	mg/mL
pH 2.2 buffer	0.007
pH 4.5 buffer	0.002
pH 6.8 buffer	0.001
FaSSIF	0.005
FeSSIF	0.016
10%HPBCD/water	0.62
Labrasol	1.22
Labrafil M 1944	0.27
Capmul MCM	3.21
Miglyol 812N	0.67
Propylene Glycol	3.43
Capryol 90	2.47
PEG 400	1.27