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Matrix effects in nilotinib formulations with pH-responsive polymer produced by carbon dioxide-mediated precipitation

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ABSTRACT

Factors determining the pH-controlled dissolution kinetics of nilotinib formulations with the pH-titrable polymer hydroxypropyl methylcellulose phthalate, obtained by carbon dioxide-mediated precipitation, were mechanistically examined in acid and neutral environment. The matrix effect, modulating the drug dissolution, was characterized with a battery of physicochemical methodologies, including ToF-SIMS for surface composition, SAXS/WAXS and modulated DSC for crystallization characterization, and simultaneous UV-imaging and Raman spectroscopy for monitoring the dissolution process in detail. The hybrid particle formulations investigated consisted of amorphous nilotinib embedded in a polymer matrix in single continuous phase, displaying extended retained amorphicity also under wet conditions. It was demonstrated by Raman and FTIR spectroscopy that the efficient drug dispersion and amorphization in the polymer matrix were mediated by hydrogen bonding between the drug and the phthalate groups on the polymer. Simultaneous Raman and UV-imaging studies of the effect of drug load on the swelling and dissolution of the polymer matrix revealed that high nilotinib load prevented matrix swelling on passage from acid to neutral pH, thereby preventing re-precipitation and re-crystallization of incorporated nilotinib. These findings provide a mechanistic foundation of formulation development of nilotinib and other protein kinase inhibitors, which are now witnessing an intense therapeutic and industrial attention due to the difficulty in formulating these compounds so that efficient oral bioavailability is reached.

1. Introduction

Protein kinases are catalysts for protein phosphorylation, controlling a number of cellular processes. Consequently, they are important pharmacological targets for the therapeutic treatment of a variety of human diseases associated with the abnormal protein phosphorylation, such as chronic myelogenous leukemia (CML), non-small cell lung cancer, and renal cell carcinoma, as well as a range of other tumors (Di Gion et al., 2011; Duckett and Cameron, 2010; van Erp et al., 2009). Notably, protein kinase inhibitors (PKIs) have developed to an important class of anti-cancer therapeutics (Experts in Chronic Myeloid and Leukemia, 2013). Among these, nilotinib is a second generation c-Abl PKI (Sabitha, 2012; Weisberg et al., 2007), with demonstrated therapeutic efficacy against drug-resident CML, philadelphia (Ph)-positive acute lymphocytic leukemia, and acute lymphoblastic leukemia (Davies et al., 2009; Manley et al., 2010; Takahashi et al., 2014). In addition, recent evidence suggests the possible application of this compound in the treatment of liver fibrosis, soft tissue sarcomas, and even for protection against neuronal degeneration (Karuppagounder et al., 2014; Shaker et al., 2013; Villar et al., 2012). Compared to first generation PKIs, nilotinib is more lipophilic and its transportation is less influenced by the interaction with transporters (Davies et al., 2009; Deadman et al., 2013; Hegedus et al., 2009). However, the poor water solubility of nilotinib also results in low-to-moderate oral bioavailability, as seen e.g., for the commercial crystalline HCl-nilotinib salt (Tasigna®) (European Medinces Agency, 2009; Tanaka et al., 2010; Xia et al., 2012).

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Therefore, the therapeutic efficacy of nilotinib is partially restrained by its solubility, which limits the exposure to the drug.

Recently, the oral bioavailability of nilotinib in dogs was demonstrated to be significantly improved upon its amorphous formulation with hydroxypropyl methylcellulose phthalate (HP55) and surfactants by carbon dioxide-mediated processing (Jesson et al., 2014). In parallel, the nano-sized formulation considerably enhanced nilotinib dissolution in simulated intestinal fluids in vitro, ultimately increasing the nilotinib blood concentration in vivo (Jesson et al., 2014). Nevertheless, important aspects of the mechanisms underlying nilotinib dissolution and re-precipitation kinetics mediated by this formulation were not clarified. These include the relative importance of drug amorphization and effects of the pH-sensitive matrix on the drug dissolution, as well as the matrix effects on re-precipitation and re-crystallization. In fact, the amorphous solid dispersions (ASD) of nilotinib in HP55 can affect the drug oral absorption through several mechanisms, including: (i) enhanced drug dissolution rate, (ii) inhibition of re-precipitation and re-crystallization of dissolved drug, and (iii) pH-controlled release of the drug (Grohganz et al., 2014; Kohri et al., 1999). ASDs can significantly increase the apparent solubility of poorly soluble molecules, while preserving their permeability (Miller et al., 2012). However, the supersaturation condition (i.e., the apparent drug solubility exceeding the saturation conditions) is thermodynamically unstable, and its magnitude and persistence greatly depend on a number of poorly controllable factors (Ozaki et al., 2013; Warren et al., 2010). Thus, it is required a strict control over supersaturation dynamics for mediating effective oral drug delivery, as discussed, e.g., by Augustijns and Brewster (2012). Specifically, fast and intense supersaturation occurring in the gastric environment is not desirable for weak bases, since this generally results in fast re-precipitation of the drug in the luminal environment (Van Speybroeck et al., 2010). Consequently, pH-controlled dissolution and super-saturation induction may have a remarkable impact on the absorption of poorly soluble weak bases, such as nilotinib (DiNunzio et al., 2008; Kohri et al., 1999; Miller et al., 2008).

In the current study, nilotinib amorphous dispersions with HP55 are further investigated, with specific focus on the interplay between the pH-controlled drug/matrix dissolution and drug re-precipitation. The aim was to clarify the physicochemical properties of this formulation type, and their role in mediating a favorable drug dissolution profile. Therefore, nilotinib formulations obtained at various drug loads for the phthalate-containing and pH-titratable HP55 were compared to the corresponding formulations obtained for non-titrating hydroxypropyl methylcellulose (HPMC). In order to focus on matrix effects only, surfactants were excluded throughout. The formulations were analyzed by a range of complementary physical techniques to reveal their physical properties and structural features. In addition, the dissolution profile as a function of drug load was studied under both acid conditions at pH 2.2 and under neutral conditions at pH 6.4, as well as during the transfer from the former to the latter, simulating the pH transfer occurring during the passage from stomach to intestine. Finally, simultaneous UV imaging and Raman spectroscopy was recorded to acquire information on the composite matrix behavior during dissolution in real-time. This comprehensive characterization of the drug-load-dependent dissolution kinetics allowed for the identification of preponderant parameters to direct further nilotinib formulation development.

2. Material and methods

2.1. Chemicals

Hydroxypropyl methylcellulose USP2910 (HPMC) was from Dow Chemical Company (Midland, USA), while hydroxypropyl methylcellulose phthalate (HP55) was from Shin-Etsu (Tokyo, JPN). Nilotinib base (99% purity) was from Hwasun Biotechnology Co. (Shanghai, CHN). Dimethyl sulfoxide (DMSO), citric acid, sodium phosphate di-basic, ammonium formate, formic acid, and additional chemicals were obtained commercially at analytical grade (Sigma–Aldrich, St. Louis, USA). Acetonitrile (LCMS grade) was from Fisher Scientific (Loughborough, UK).

Citrate-phosphate buffer (referred simply as “citrate”) at pHs 2.2 and 6.4 was prepared by mixing citric acid (0.1 M) and sodium phosphate di-basic (0.2 M) at 49:1 and 0.44:1 (v/v) ratio, respectively.

2.2. Hybrid nilotinib/HP55 particle preparation

Nilotinib base (crystalline) was formulated by carbon dioxide-mediated processing with HP55 at a drug load of either 20% (w/w; DL20) or 40% (w/w; DL40), in absence of surfactant, as previously reported (Jesson et al., 2014). In short, the carbon dioxide-mediated processing system used for hybrid nanoparticle generation consists of one pumping set-up for the solution containing the active ingredient (nilotinib dissolved in a solvent, together with either HP55 or HPMC) and one for the anti-solvent (CO_2). The two set-ups are connected at a nozzle where particles are produced by anti-solvent precipitation. Particles are retained in the reactor by a filtering set-up. A back-pressure regulator is used after the reactor for pressure control. Each pumping set-up is equipped with separate flow and pressure meters for good process control. Before particle generation, CO_2 and solvent are pumped through the system (at 100 g/min and 1 ml/min, respectively) until flow rates, pressure (125 bar), and temperature (15 °C) have reached steady state. The solvent is then replaced by solvent containing nilotinib and polymer, and nanoparticles are produced in the reactor. After completion of pumping of this solution, CO_2 is pumped through the reactor in order to extract residual solvent from the reactor and the powder. Finally, the reactor is depressurized, and the sample collected. Quality control on the batches was routinely performed by HPLC to verify the drug purity (data not shown). The formulations obtained were assessed before use by X-ray power diffraction (XRD) and scanning electron microscopy (data not shown), to verify that particle amorphousness and size in was in accordance with the previously reported data (Jesson et al., 2014). A similar process was used to formulate nilotinib with HPMC at drug load 20% and 40% w/w. The physical mixes (MMX) were prepared mixing 20–40% w/w nilotinib with HP55.

2.3. Liquid chromatography–mass spectrometry (LC–MS)

The chromatographic system used was an Acquity UPLC i-Class system equipped with a BEH C18 1.7 μm, 2.1 × 50 mm column, all from Waters Corporation (Milford, USA). Mobile phase A consisted of 1 mM ammonium formate in Milli Q water, adjusted to pH 3.0 with formic acid, while mobile phase B consisted of acetonitrile. 5 μl of sample were injected and run at a linear gradient from 10 to 45% B over 5 min, followed by 2 min re-equilibration time at a flow rate of 0.3 ml min^{-1}. The LC-system was connected to a Quattro Micro mass spectrometer from Waters Corporation (Milford, USA), equipped with an electrospray ionization source operated in positive mode. Nilotinib quantification was performed in single ion monitoring (SIM) mode, using the $(M+H)^+$ ion at 530.3 m/z. For degradation products, the MS was set to scanning the 200–600 m/z region (Kondra et al., 2014).

2.4. Wide- and small-angle X-ray scattering (WSAXS)

Samples containing 100–2000 mg/ml of nilotinib in citrate buffer, pH 2.2 and 6.4 (corresponding to sample:buffer ratios of