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In vitro characterization of ritonavir formulations and correlation to in vivo performance in dogs

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ABSTRACT

Ritonavir (RTV) is a weakly basic drug with a pH-dependent solubility. In vitro characterization of dissolution and supersaturation behaviors of three PEG-8000 based amorphous solid dispersions (ASD) and a physical blend (PB) with crystalline drug were performed in the biomimetic media (e.g., FaSSGF, FaSSIF, FaSSIF-V2). A two-stage dissolution test and a biphasic dissolution-partition test at the small scale (referred as to biphasic test) were employed with intention to examine the in vitro and in vivo relationship (IVIVR) with retrospective PK data in dog model.

The two-stage dissolution test revealed a high degree of supersaturation of RTV from these ASDs accompanied by the occurrence of liquid–liquid phase separation (LLPS) in the biomimetic media. A rapid decrease of apparent RTV concentrations of these ASDs was associated with significant precipitation upon the pH shift of the dissolution medium, revealing the important role of "the gastric stage". In comparison, the biphasic test revealed a lower degree of supersaturation of RTV that is attributed to removal of RTV through partition into octanol, acting as "the absorption compartment". These two dissolution tests provide characterization of the supersaturation state with a complex, dynamic interplay among dissolution, precipitation and partition processes. Results of both in vitro dissolution tests are in good agreement with in vivo results in dogs. In addition, three commercial generic RTV drug products were examined by the biphasic test. Agreement was also obtained between the RTV concentrations in octanol at 3 h from these generic drug products and their corresponding relative bioavailability in dogs.

1. Introduction

The design of supersaturation sustaining formations as an effective strategy to enhance the in vivo absorption drugs with low aqueous solubility has been extensively explored in the last decade (Gao and Morozowich, 2005; Brouwers et al., 2009; Frank et al., 2014a). Characterization of in vitro dissolution, and, in particular, characterization of the supersaturated state associated with these enabling formulations (e.g., amorphous solid dispersions), are highly desirable in order to optimize their bioperformances (Baghel et al., 2016l; Newman et al., 2012; Gao and Shi, 2012; Kostewicz et al., 2014; Nguyen et al., 2017; Lu et al., 2017). Diverse two-compartment dissolution methods with pH change have been utilized to simulate the dissolution and transit of dosage forms from the stomach to the small intestine in vivo (Kostewicz et al., 2014; Nguyen et al., 2017; Lu et al., 2017; Tsume et al., 2017). In contrast, a two phase dissolution-partition test method with pH shift, referred to as the biphasic test, has been developed in order to simulate a dynamic environment in which three processes of drug dissolution, precipitation and partition occur simultaneously (Gao et al., 2016; Pestieau and Evrard, 2017). A practical biphasic system combining an USP IV apparatus (with a flow cell) and an USP II dissolution apparatus was first reported by Gao and his co-workers in 2009 (Vangani et al., 2009). The biphasic test method permits the dissolution of the drug product in various aqueous media under a non-sink condition and simultaneous partition of the dissolved drug into an organic phase that acts as an "absorption compartment". The partition of the drug is driven by its free drug fraction in the aqueous phase, mimicking the absorption in vivo. Therefore, the concentration-time profile of the drug in the organic phase may serves as an output for establishing in vitro and in vivo relationships (IVIVR). Applications of the biphasic method to BCS II drugs including AMG517 (Vangani et al., 2009), celecoxib (Shi et al., 2010), ABT-072 (Shi et al., 2016), fenofibrate (Xu et al., 2018), and related formulations have been reported with IVIVR. In addition, multiple investigations of biphasic tests for several poorly water soluble drugs by other research groups demonstrated its broad utility for drug product development (Pestieau and Evrard, 2017; Frank et al., 2014a,

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2014b; Locher et al., 2016; Locher et al., 2016).

Amidon et al. developed a mechanistic model to describe the drug transport phenomenon associated with the two-phase dissolution system (Mudie et al., 2012). This approach assumes a first-order absorption kinetics and a relatively high fraction absorbed in vivo (F_a). The kinetics of drug partitioning from the aqueous phase to the organic phase is based on simultaneous equilibria and mass transfer of dissolved drug through the water-organic interface. The in vitro partitioning rate coefficient, k_p (equal to $(A_I/V_a) * P_I$; where A_I is the surface area of the interface, V_a the volume of dissolution medium, and P_I the partition coefficient through the interface), reflects the rate of drug partitioning into the organic phase. The physiological relevance of this test method is that the in vitro k_p approximates the in vivo absorption rate coefficient, k_a as described below.

$$k_p = \left(\frac{A_I}{V_a} P_I\right)_{\text{in vitro}} = k_a = \left(\frac{A}{V} P_{\text{eff}}\right)_{\text{in vivo}}$$

where A is the surface area for absorption in vivo, V the volume of the GI fluid, and P_{eff} the permeation coefficient through the membrane. Based on a known (or estimated) k_a and observed (or estimated) P_I, one can adjust A_I/V_a so that k_p and k_a become similar or equal. The biphasic test employed in our laboratory possesses a flexibility of different scaling factors (A/V) associated with the small and large scales intended to mimic different physiologies of dogs and humans. As we have reported the application of the biphasic test methods to characterize varying formulation technologies of an weak acid drug ABT-072 of extremely low solubility, good agreements were obtained between the in vitro profiles at both *small* and *large* scales and in vivo PK results in dogs and humans, respectively (Shi et al., 2016).

Oral drug delivery is a highly complex field with many unknowns. Animal models have been widely used for preliminary evaluation of new chemical entities before reaching the patient in a clinical setting—namely, in terms of safety and minimizing toxicity, but also in enabling optimization of formulation characteristics and specific drug targeting, among others. Elucidating the mechanisms of drug absorption following oral administration has remained a long-standing goal across a multitude of scientific disciplines, namely, for the purposes of predicting human safety as well as pharmacokinetics and pharmacodynamics for potential drug candidates. It has been well recognized that animal models remain a poor simulacrum of human physiology, given that many species are often fundamentally distinct in terms of their GI anatomy and physiology as compared with humans. Nonetheless, they have been frequently put to use in preclinical studies, justified by a similarity to humans by key parameters, such as GI fluids with pH variation, the transit times and/or the regional absorption.

Although significant progress has been made in recent years to improve in vitro techniques for assessment of drug characteristics and bioperformance, these approaches are not considered a reliable substitution of in vivo evaluation. Indeed, there remain significant gaps in our knowledge of animal and human physiologies, yet the animal models continue to be used in both academic and industrial environment in drug development and preclinical studies. Development of biomimetic in vitro test methods has been partially supported by reliance on animal models as intermediary models for evaluation of compounds through in vitro–in vivo correlations. Valuable information of specific physiological and anatomical parameters influencing drug absorption, especially the complex dynamics from the gut, is difficult to simulate in vitro. Such understanding from animal models enables extrapolation to humans, difficult to obtain otherwise. As the dog model still plays an important role in drug product development, it is desirable to develop appropriate in vitro tests and examine their relevance to in vivo performance in dogs.

RTV (its molecular structure shown in Fig. 1A) is a HIV protease inhibitor. It is a very weak base with pKa values of 1.8 and 2.6 (Law et al., 2001; Law et al., 2004). The crystalline Form I and II possess a high aqueous solubility at pH < 1 and extremely low solubility of 2 μg/mL at pH 4–7 (Fig. 1B). RTV was revealed to have a low to moderate intestinal permeability and is a Pgp substrate with moderate to high efflux ratio indicated by Caco-2 results (Law et al., 2004; Aungst et al., 2000; Alsenz et al., 1998). Clinical evaluation results of RTV oral exposure with respect to the effect of Pgp and MRP1 expression in HIV infected human subjects are consistent with this assessment (Meaden et al., 2002). Therefore, RTV is classified as a BCS IV drug (Law et al., 2004). It is of interest to note that RTV showed a high bioavailability of ~95 to 100% in dogs at the dose of 5 to 10 mg/kg (Kempf et al., 1995; Hsu et al., 1998). Although the absolute bioavailability of RTV in human subjects has not been determined, the fraction of dose absorbed at the dose of 600 mg was estimated to be in the rage of 60 to 80% (Kempf et al., 1995; Hsu et al., 1998). Such a high bioavailability of RTV was hypothesized due to either saturation of the Pgp efflux pump (Law et al., 2004) or other factors in vivo that minimized the active transporter effect (Alsenz et al., 1998).

Prototype PEG-8000 based ASD formulations containing RTV of 10%, 20% and 30% (*w*/w), and one physical blend of crystalline drug and PEG polymer (Table 2) were previously examined in dogs under the fasting state (Law et al., 2004). That study applied a dissolution test under a sink condition and did not investigate the supersaturation behaviors of RTV from such ASD formulations. Commercial RTV products under the brand name of Norvir® (AbbVie) including the solution (80 mg/mL), tablet (100 mg strength), and powder (100 mg/unit) were examined by two biomimetic in vitro tests including the two-stage dissolution test and the biphasic test at a large scale (Xu et al., 2017). This was to characterize dissolution and precipitation behaviors of RTV in the aqueous media and establish their relevance to pharmacokinetic results in human subjects. These results revealed that the relationship between supersaturation and solubilization of RTV from related PVP-VA based ASDs is complex and highly media dependent. In addition to achieve an agreement of the in vitro-in vivo correlation (IVIVC), we gained mechanistic understanding of the food effect from the tablet and powder formulations observed in vivo (Xu et al., 2017).

Relative bioavailability of RTV products manufactured by various generic pharmaceutical companies was examined against the commercial Norvir® capsule using an internally developed dog model at Abbott/AbbVie (Garren et al., 2010). Although some commercial generic RTV drug products were selected for evaluation in dogs, they have not been examined by the biomimetic in vitro tests at AbbVie.

The purpose of this work was to (1) evaluate RTV prototype PEG-8000 ASDs by two biomimetic in vitro tests including the two-stage dissolution test and the biphasic test and (2) evaluate three commercial generic RTV drug products manufactured by Emcure, Hetero and Cipla. This study was aimed to assess the utility of biomimetic in vitro test methods and provide mechanistic understanding of the dynamic relationship among the dissolution, precipitation and partition kinetics of RTV in the aqueous media. These tests were evaluated to examine the relevance of in vitro profiles to the in vivo exposure of all RTV formulations tested in dogs.

2. Materials and Methods

2.1. Materials

Crystalline RTV was obtained in house. Poly(ethylene glycol) 8000 (PEG-8000, Carbowax) was obtained from Dow Chemical Company (USA). Ethanol, octanol, acetonitrile, maleic acid, sodium hydroxide, acetic acid, sodium acetate and sodium chloride were purchased from Sigma (St. Louis, MO). The FaSSGF. FaSSIF and FaSSIF-V2 powders were purchased from Biorelevant (UK).

2.1.1. ASD Formulations and Generic Drug Products

The preparation of the ASDs containing 10%, 20%, and 30% in PEG-8000 matrix and 10% physical mixture (PB) containing crystalline RTV