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Non-Sink Dissolution Behavior and Solubility Limit of Commercial Tacrolimus Amorphous Formulations

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ABSTRACT

An increasing number of drugs with low aqueous solubility are being formulated and marketed as amorphous solid dispersions because the amorphous form can generate a higher solubility compared to the crystalline solid. The amorphous solubility of a drug can be determined experimentally using various techniques. Most studies in this area investigate the drug in its pure form and do not evaluate any effects from other formulation ingredients. In this study, we use 6 marketed amorphous oral drug products, capsules containing 5 mg of tacrolimus, and various excipients, consisting of 1 innovator product and 5 generics. The amorphous solubility of tacrolimus was evaluated using different techniques and was compared to the crystalline solubility of the drug. Dissolution of the different products was conducted under non-sink conditions to compare the maximum achieved concentration with the amorphous solubility. Diffusion studies were performed to elucidate the maximum flux across a membrane and to evaluate whether there was any difference in the thermodynamic activity of the drug released from the formulation and the pure drug. The amorphous solubility of tacrolimus was found to be a factor of 35 higher than the crystalline solubility. The maximum concentration obtained after dissolution of the capsule contents in non-sink conditions was found to match the experimentally determined amorphous solubility of the pure drug. Furthermore, the membrane flux of tacrolimus following dissolution of the various formulations was found to be similar and maximized. This study demonstrates a link between key physicochemical properties (amorphous solubility) and *in vitro* formulation performance.

Introduction

Increasingly, many of the new drugs being discovered are poorly water soluble,[1] and hence the formulation of these compounds into dosage forms that result in increased water solubility has become a critical issue.[2] There are several strategies to increase solubility including salt or cocrystal formation,[3-5] micellar solubilization,[6,7] complexation with cyclodextrins,[8,9] and formulating into amorphous solid dispersions (ASD).[10,11] Salt formation is only possible for compounds with acidic or basic groups whereas cocrystal formation with a hydrophilic co-former can be potentially used for neutral compounds. Both salts and cocrystals can lead to supersaturated solutions[12-14] which can subsequently crystallize to the free form of the drug. In contrast, micellar solubilization and cyclodextrin complexation increase the equilibrium solubility of the crystalline drug without supersaturation,[15,16] and hence crystallization is less of a concern. However, the rate of passive absorption depends on the free drug concentration, hence increasing the solubility in this manner does not necessarily lead to an enhanced membrane transport rate.[17] Amorphization increases the chemical potential of the drug, resulting in the formation of a supersaturated solution following dissolution; supersaturation is generated under non-sink dissolution conditions in the absence of crystallization.[17,18] Therefore, for ASD formulations, it is important to test the *in vitro* dissolution behavior under non-sink conditions to evaluate the ability of the formulation to generate and maintain supersaturation because this can affect the *in vivo* performance.[19] One drawback is that the higher free energy of the amorphous material relative to the crystalline state results in a driving force for crystallization, and hence the physical stability of the solid is a practical concern. Therefore, the addition of polymers to form and stabilize an ASD becomes

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important because this leads to amorphous formulations with the required physical stability.[20] The longevity of the supersaturated solution after dissolution is also another challenge because crystallization from the solution phase can be very rapid. Once again, certain polymers are useful formulation components that can reduce solution crystal nucleation and growth rates, leading to sustained supersaturation and improvements in bioavailability relative to crystalline forms.[21] Due to the success of amorphous formulations at improving bioavailability, there have been a number of products based on this formulation approach that have been commercialized, in particular in the past few years, with examples including products such as Sporanox®, Zelboraf®, and Norvir®.

One of the earliest commercial ASD products contains tacrolimus and was introduced by Fujisawa Pharmaceuticals (now Astellas Pharma). Tacrolimus, also called FK-506, is a potent macrolide compound (with a molecular weight of 804 g/mol) produced by *Streptomyces tsukubaensis* and is used as an immunosuppressive agent in organ transplant patients to prevent organ rejection.[22] Capsule formulations containing an ASD of tacrolimus (0.5, 1, and 5 mg) were first approved by the U.S. Food and Drug Administration in 1994 and was introduced under the trade name Prograf®. Since then, there have been several generic tacrolimus products introduced to the market. In this case, it is essential that the physicochemical characteristics (e.g., maximum achievable supersaturation, product stability) of generic formulations be comparable to the brand-name formulation in order to ensure the brand-generic bioequivalence and therapeutic substitutability throughout the product shelf life.[23] Crystalline tacrolimus is a monohydrate with a reported aqueous solubility of 0.7-1.3 ug/mL.[15,24] Human pharmacokinetic studies of tacrolimus have shown that it has a narrow therapeutic window and that absorption is highly variable between individuals, with peak concentrations being achieved between 0.5 and 6 h with a mean bioavailability of 25% in transplant patients.[25] There are various factors that can affect tacrolimus oral bioavailability including its low water solubility, site-dependent permeability, intestinal and first-pass metabolism,[26] drug efflux,[27] and simultaneous food and medication intake.[28] Tacrolimus is a substrate for P-glycoprotein and also for the cytochrome P450 which decreases blood concentrations via metabolism pathways.[29,30] The crystalline form is known to have a low oral bioavailability whereas the ASD, in contrast, improves the oral absorption, leading to an order of magnitude improvement in the area under the curve in the plasma-time concentration profile, when evaluated in beagle dogs.[31]

In previous studies with various drugs, we have found that there is a maximum concentration of molecularly dissolved drug that exists in a supersaturated solution and that this concentration is dictated by the amorphous solubility. If this concentration is exceeded, and crystallization does not occur, liquid-liquid (or glass-liquid) phase separation (LLPS) takes place with the formation of a new phase.[32] This phase is a water-saturated colloidal amorphous phase that can either be a supercooled liquid or a glass depending on the glass transition temperature of the precipitate. Although this behavior has been extensively documented for supersaturated solutions created by antisolvent addition, there are only a few studies where the solution-phase behavior of model ASD systems has been evaluated in terms of their tendency to undergo LLPS during dissolution. Furthermore, to the best of our knowledge, there are no reports of the supersaturation and solution-phase behavior of commercial formulations, which are more complex because they contain both the ASD particles and other excipients. In particular, it is of interest to determine whether the supersaturation behavior of a commercial ASD formulation can be predicted based on the determination of key parameters such as the compound amorphous solubility, and crystallization kinetics from a simple solution. The hypothesis is that the maximum free drug concentration (supersaturation) that can be generated by the dissolution of commercially available tacrolimus formulations is dictated by the amorphous solubility of tacrolimus. To test this hypothesis, the dissolution profiles of 6 commercially available tacrolimus capsules (i.e., 1 innovator and 5 generic products) were evaluated under non-sink dissolution conditions. The thermodynamic properties of the resultant solutions were then evaluated using flux measurements. Experiments were conducted to probe the following questions: (1) is crystallization detected during non-sink dissolution? (2) how similar is the dissolution of the various tacrolimus products manufactured by different companies? (3) are these commercial formulations able to dissolve to reach the amorphous solubility, and (4) is the amorphous solubility exceeded leading to the formation of a new colloidal phase?

Materials

Pure tacrolimus monohydrate was purchased from Attix Pharma (Toronto, Canada). Hydroxypropyl methylcellulose (HPMC) was a gift from Shin-Etsu chemicals (Niigata, Japan). Cros-carmellose sodium (CCS-Na) was purchased from FMC Biopolymer (Newark, DE). Lactose monohydrate was obtained from Spectrum Chemicals (Gardena, CA). The different brands of marketed 5-mg tacrolimus capsules were purchased from the Purdue Pharmacy. The manufacturers and the lot numbers of the formulations are as follows: Astellas Pharma (Lot no. 047354), Mylan (Lot no. 3057525), Panacea Biotech (Lot no. 4233501), Dr. Reddy's (Lot no. C402194), Intas Pharma (Lot no. R09717), and Sandoz (Lot no. 047354). At the time of analysis, all products were within their expiration date.

The dissolution medium, which consisted of 5 mM pH 4.5 buffer, was prepared by dissolving 685 mg of monosodium dihydrogen phosphate monohydrate in 1 L of water and acidified to pH 4.5 with 6% phosphoric acid.

Methods

Thermal Analysis

Crystalline tacrolimus monohydrate samples were analyzed using a differential scanning calorimeter (DSC) model Q2000 (TA Instruments, New Castle, DE). The instrument was calibrated for temperature using indium and tin and for enthalpy using indium. Dry nitrogen at 50 mL/min was used as the purge gas. Around 5 mg of tacrolimus powder was placed in the sealed sample pan with a pinhole lid. The sample was heated to 160°C until the sample melted and then cooled at 15°C/min to 0°C and then reheated at 10°C/min to determine the glass transition and stability of the melted solid to recrystallization.

Spectroscopic Analysis of the Different Capsule Contents

Fourier transform infrared (FTIR) spectra of the different samples were obtained using a Bruker Vertex 70 (Bruker Optics Inc., Billerica, MA). And 128 scans were recorded for each sample over the spectral region 600-4000 cm^{-1}. The samples were scanned using a Golden Gate Bridge attenuated total reflectance accessory from Specac Instruments (Swedesboro, NJ). FTIR spectra of the excipients present in the formulations were also recorded.

Solubility Determination

Crystal solubility of tacrolimus monohydrate was determined by adding an excess amount of tacrolimus monohydrate to pH 4.5