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The fate of ritonavir in the presence of darunavir

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ABSTRACT

This study was the first investigation into the potential of a fixed dose combination of ritonavir and darunavir in the form of dispersible powders prepared by spray drying. A common polymer (hydroxypropyl methylcellulose, polyvinylpyrrolidone, and polyvinylpyrrolidone-vinyl acetate 64) was formulated with either ritonavir or darunavir or a combination of ritonavir and darunavir. The influence of these polymers on the supersaturation level of ritonavir and darunavir was investigated. The concentration levels of ritonavir and darunavir during these tests dropped instantly to a plateau which could be considered as amorphous solubility. Besides, the presence of darunavir always decreased the supersaturation level of ritonavir and vice versa no matter which polymers were used. Moreover, the rate and extent of release of both ritonavir and darunavir from ternary spray-dried powders were less than the releases from binary spray-dried powders. Intermolecular interaction between ritonavir and darunavir was ruled out by ^{1}H NMR study which means that the decrease in supersaturation level or release must be at least partially attributed to the mediated solvent process. In order to restrict the mutual influence between darunavir and ritonavir, a complex of both ritonavir and darunavir with (2-hydroxypropyl)-β-cyclodextrin was prepared and improved the dissolution rate of both ritonavir and darunavir.

1. Introduction

An increasing number of infants and children become infected with human immunodeficiency virus (HIV), most commonly through mother-to-child transmission of the virus (WHO and UNICEF, 2011). In 2011, it was estimated that approximately 3.3 million children less than 15 were living with HIV infection and 10% of them were newly infected, especially in developing countries (WHO and UNICEF, 2012). The high mortality rate associated with pediatric HIV infection highlights the vulnerability of this neglected population. This is partly due to the difficulty in providing antiretroviral therapy (ART) for children and infants in such population. Several reasons could be accountable for the fact that pediatric HIV-treatment coverage is lagging far behind that of adults. Firstly, in terms of drug formulation and doses, the current available oral dosage forms like tablets or capsules cannot enable accurate dosing for children who need flexible dosage regimen according to age, weight or other parameters that change with growth (Zajicek et al., 2013). Secondly, ART is a lifelong treatment and its complexity which normally requires taking a combination of anti-HIV medications (a regime) everyday makes it difficult not only for pediatric patients but also for adults to strictly follow the ART regimes (Bennett et al., 2008). Lastly, although there is a considerable effort to develop and introduce new active pharmaceutical ingredients (APIs) and therapies, most of the current approved ART involve poorly soluble APIs which result in their limited and variable bioavailability (Van Heeswijk et al., 2001). All of the above reasons contribute to the advent of the resistant HIV type 1 and consequently cause the failure of ART. That is why any drug regimens that are simple, effective, low in toxicity, easily accessible, highly insusceptible to drug resistance and offer convenience in terms of use should be developed and promoted.

According to a recently WHO dosing guidance for formulations of pediatric ART, a combination of ritonavir (RTV) and darunavir (DRV) for the second line HIV-treatment is recommended (WHO, 2011). It is actually a classic regimen based on a protease inhibitor (PI), boosted where possible with RTV. In this regime, DRV, a second-generation PI, is commonly indicated for the treatment in patients with established resistance to other drugs in this class (US Department of Health and Human Services, 2013a). Meanwhile RTV is mainly used as a PI booster because of its potent inhibition of the cytochrome P450 3A4 (CYP3A4) isoenzyme that metabolizes other PIs, thereby prolonging the half-life and increasing drug exposure of other co-administered PIs (US Department of Health

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and Human Services, 2013b). Although a RTV/DRV fixed dose combination was recently approved by the US Food and Drug Administration, RTV/DRV is not currently available as a co-formulated fixed dose combination for children.

In this study, we explore the feasibility of an "advanced" fixed dose combination of DRV and RTV i.e., a single powder made up of RTV and DRV and excipients, comparable to the "ready-to-use" excipients mix known for direct compression purpose like MicroceLac® 100 (Michoel et al., 2002), prepared by spray drying. In spite of the flowability issues, fixed dose combination powders can give more convenience to patients in general and for children in particular. Moreover, the spray-dried powders offer some other advantages. During the spray-drying process, the amorphization of drugs leads to higher solubility because of the higher free energy of the amorphous state compared to the crystalline counterpart (Paudel et al., 2013). Also, the knowledge acquired with respect to the formulation and processing of spray-dried powders can be transferred to an actual pediatric formulation such as powders for reconstitution which is an attractive pediatric dosage form. The powders can be reconstituted to drinkable solutions or sprinkle formulations and the administered dose can be easily adapted to the need of the pediatric patients. Although other drinkable formulations (syrups or solutions) can also offer flexible dosage regimen, spray-dried powders have their advantages over liquid dosage forms in terms of compactness, storage, stability and transport (Van Gyseghem et al., 2008). More importantly, because of the higher free energy of APIs in amorphous state, upon exposure to water, a supersaturated drug solution will be generated which enhances the absorption and consequently bioavailability. However, as the chemical potential of a supersaturated system is increased compared to that of a saturated condition, supersaturation is inherently thermodynamically unstable. The process such as nucleation and crystal growth (i.e., precipitation) allow them to reach a more stable state (Lindfors et al., 2008) that eventually nullifies the supersaturation's benefits. That is why all formulations based on creating supersaturating systems requires various pharmaceutical excipients that can prolong supersaturation (Brouwers et al., 2009). Therefore, in the first part of this study, we investigate the ability of various polymers in maintaining supersaturation of both RTV and DRV as well as the influence of RTV on the supersaturation profile of DRV and vice versa. In the second part, a series of formulations of both RTV and DRV are co-spray dried into fixed dose combination with cyclodextrins which is widely used as complexing agents to increase the aqueous solubility of drugs.

2. Materials and methods

2.1. Materials

Ritonavir (RTV) was obtained from Pharmidex (London, UK). Darunavir (DRV), in the form of darunavir ethanolate was a gift from Cilag AG (Schaffhausen, Switzerland). Hydroxypropyl methylcellulose 2910 5 mPa s (referred as HPMC afterward) was provided by Colorcon (UK). Polyvinylpyrrolidone (PVP K30) and polyvinylpyrrolidone-vinyl acetate 64 (PVPVA 64) were provided by BASF ChemTrade GmbH (Ludwigshafen, Germany). (2-Hydroxypropyl)-β-cyclodextrin (HP-β-CD) was a generous gift from Janssen Pharmaceutica (Beerse, Belgium) and sulfobutyl ether-β-cyclodextrin (SBE-β-CD) was purchased from CyDex Inc. (Kansas, US). Methanol and acetonitrile (HPLC grade) were obtained from Acros Organic (Geel, Belgium). Other chemical reagents were of analytical grade. In all the experiments deionized water (Maxima Ultra Pure Water, Elga Ltd., Wycombe, England) was used.

2.2. Equilibrium solubility measurement

Equilibrium solubilities of RTV and DRV were determined in water, HCl 0.02 M (pH 1.69 ± 0.02) as well as in pre-dissolved polymer solutions (PVP K30, PVP VA64, and HPMC at 0.2 mg/ml) ($n = 3$). In a glass test tube, an excessive amount of pure RTV or DRV was added into 10 ml of the above media and then the test tube was rotated by using a rotary mixer L26 (Labinco BV, Breda, The Netherlands) for 72 h. After that, 1 ml of the sample was filtered through a PTFE membrane with 0.45 μm pore size (Grace Davison Discovery Science, Illinois, USA). Drug adsorption to the filter membrane was ruled out in preliminary experiments. The content of dissolved RTV and DRV in the filtrate was determined by using reversed phase HPLC (Merk-Hitachi LaChrom system) under the following set of conditions: ODS Hypersil C18 column (150×4.6 mm 5 μm) (Thermo Scientific, USA), the mobile phase was a mixture of 55:45 (v/v) acetonitrile: 10 mM sodium dihydrogen phosphate solution (adjusted to pH 4.8 with 1 M NaOH). The flow rate and the injection volume were 1 ml/min and 20 μl, respectively and the UV detection was performed at 245 nm. A standard calibration curve (peak area vs. known concentration) was developed by using standard solutions (5–100 μM) prepared by diluting the stock standard solution (RTV and DRV in dimethyl sulfoxide (DMSO)) with the mobile phase. Linearity was confirmed through linear regression analysis which gave a determination coefficient value (R^2) of ≥ 0.999.

2.3. Precipitation/supersaturation studies

The purpose of the test is to investigate the effect of polymers on maintaining the supersaturation of RTV and DRV in solution. The supersaturation studies were carried out using two different systems. In the first part of the studies, a USP II Dissolution Apparatus was used whereas in the second part, a rotary mixer was used. Basically, supersaturated solutions were created using solvent shift method. A series of polymer solutions with a concentration of 0.2 mg/ml was prepared by dissolving a predetermined amount of the polymers (PVP K30, PVP VA64 and HPMC) in water or HCl 0.02 M. The testing procedure applied for the two systems was as follows: 1/In a dissolution vessel, 5 ml of single RTV or DRV solution or 5 ml of a solution composed of both RTV and DRV in DMSO at concentration of 10 mg/ml and 60 mg/ml, respectively was added into 500 ml of the polymer solution. The resultant solutions were then stirred at 100 rpm using a USP II Dissolution Apparatus (SR8 Plus, Hanson Research, USA) at 37 ± 0.5 °C. 2/In a glass test tube, 100 μl of the same RTV and DRV solutions were added into 10 ml of the polymer solution and the test tube was rotated using a rotary mixer at room temperature. During the experiment, 1 ml of sample was taken at different time intervals (5, 15, 30 min, 1, 2, 4 and 8 h), then filtered through a PTFE membrane with 0.45 μm pore size (Grace Davison Discovery Science, Illinois, USA). The content of RTV and DRV in the filtrate was determined by using reversed phase HPLC as describe above.

2.4. Polarized light microscopy (PLM)

Polarized light microscopy was used to monitor physical characteristics of the precipitates such as morphology and crystallinity during the precipitation test. In a 12-glass-well plate, a small volume (1 μl) of a highly concentrated solution of RTV and/or DRV in DMSO was added to 99 μl water to induce the same degree of supersaturation as in the precipitation test described above. Afterwards, the wells were sealed immediately with a glass coverslip using silicone grease to prevent evaporation of water. The samples were then visualized for the presence of birefringence as