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Research Paper

Sirolimus formulation with improved pharmacokinetic properties produced by a continuous flow method

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ABSTRACT

The oral bioavailability of Sirolimus is limited by poor dissolution of the compound in the gastrointestinal tract resulting in a low bioavailability and large inter-individual differences in blood levels. Several different formulation approaches were applied to overcome these disadvantageous pharmacokinetic properties including the marketed oral solution and a tablet form containing wet milled nanocrystals. These approaches deliver improved pharmacokinetics, yet, they share the characteristics of complex production method and composition. We have developed a nanostructured Sirolimus formulation prepared by the controlled continuous flow precipitation of the compound from its solution in the presence of stabilizers. We have shown that contrary to the batch production the process could be easily intensified and scaled up; apparently the uniformity of the precipitation is heavily dependent on the production parameters, most likely the mixing of the solvent and antisolvent. We compared the physicochemical and pharmacokinetic properties of the nanostructured formula with the marketed nanoformula. We found that our method produces particles in the size range of less than 100 nm. The solid form redispersed instantaneously in water and in biorelevant media. Both the solid form and the redispersed colloid solution showed excellent stability even in accelerated test conditions. The oral administration of the nanostructured formula resulted in faster absorption, higher exposure and higher trough concentrations when compared to the marked form. These advantageous properties could allow the development of solid oral Sirolimus formulae with lower strength and gel based topical delivery systems.

1. Introduction

Sirolimus is a macrocyclic lactone immunosuppressant, indicated for the prophylaxis of organ rejection in patients receiving renal transplants. Its mechanism of action is distinct from other immunosuppressive agents as it binds to cytosolic FK-binding protein 12 (FKBP12) to generate an immunosuppressive complex [1]. It is a white to off-white crystalline solid with a molecular weight of 914.17. The chemical structure of the compound is shown in Fig. 1. It is practically insoluble in water, freely soluble in benzyl alcohol, chloroform, acetone and acetonitrile, at least soluble in methanol and tetrahydrofuran, sparingly soluble in ethanol and isopropanol and slightly soluble in n-propanol. Sirolimus has a log *P* value of 4.3 at pH 7.0 and possesses very low aqueous solubility (2.6 μg/ml) [2]. The low solubility of the compound is generally accepted to be one of the rate limiting factors of oral absorption [3]. The oral bioavailability of the compound is further limited by multiple active processes which include metabolism by CYP3A4 and transport by PgP [4].

In order to improve oral absorption of the compound first an oral solution was developed. It is marketed under the trade name Rapamune® oral solution. Later, a milled nanocrystal formula was developed which allowed the development of a tablet form marketed as Rapamune® tablet available in 0.5 mg, 1 mg and 2 mg strengths. The inactive ingredients in Rapamune® Oral Solution are Phosal 50 PG® (phosphatidylcholine, propylene glycol, mono- and di-glycerides, ethanol, soy fatty acids, and ascorbyl palmitate) and polysorbate 80. It also contains 1.5–2.5% ethanol. The inactive ingredients in Rapamune® tablets include sucrose, lactose,

Abbreviations: AUC, area under the curve; API, active pharmaceutical ingredient; SRL, Sirolimus; CYP, cytochrome P450; PgP, P-glycoprotein; FaSSIF/FeSSIF, Fasted/Fed State Stimulating Intestinal fluid; PAMPA, parallel artificial membrane permeation assay; SDS, sodium dodecyl sulfate; RT, room temperature; RH, relative humidity.

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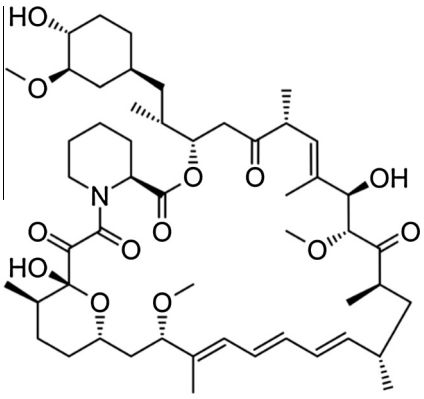

Fig. 1. The chemical structure of Sirolimus.

polyethylene glycol 8000, calcium sulfate, microcrystalline cellulose, pharmaceutical glaze, talc, titanium dioxide, magnesium stearate, povidone, poloxamer 188, polyethylene glycol 20,000, glyceryl monooleate, carnauba wax, dl-alpha tocopherol, and other ingredients [3]. The above list of stabilizers, solubilizers and excipients along with the complicated wet-milling process that was utilized for the development of the tablet clearly indicates the complexity of the problem of efficient oral delivery of Sirolimus.

Despite utilizing these multi-component delivery systems Sirolimus is absorbed from the intestine variably, with some patients having up to eight times higher exposure than others for the same dose [5]. The systemic availability of Sirolimus is low, and was estimated to be approximately 14% after the administration of Rapamune® Oral Solution. In healthy subjects, the mean bioavailability of Sirolimus after the administration of the tablet is approximately 27% higher relative to the solution [6,7]. Therefore, the oral solution is not bioequivalent to the tablet form. At the same time the precise control of Sirolimus blood concentrations is crucial. Although contrary to Cyclosporine A no significant renal toxicity was reported in case of high blood concentrations [8,9], and similar to Cyclosporine A low trough concentrations are associated with higher incidence of organ transplant rejection [6,9]. To minimize the variability in Sirolimus blood concentrations, both Rapamune® Oral Solution and tablets should be taken consistently with or without food once daily. Drug trough levels are taken once daily before the administration of the next dose [6]. Trough levels were found to correlate with overall exposure well [9].

Topical application of Sirolimus was also attempted [10]. A wide range of indications from psoriasis to skin cancer has been proposed as potential targets of this administration route. Given the very low water solubility of Sirolimus the active is administered topically as petrolatum or emulsion type creams, or as the same Rapamune® solution used for oral treatment [11].

Besides the marketed nanoformula other attempts have also been made to use nanotechnology to circumvent the very low water solubility of Sirolimus. Porous carriers [12] and polymeric nanoparticles using N-isopropylacrylamide, methylmethacrylate and acrylic acid in situ polymerization technique [13] have been developed. Sirolimus-beta cyclodextrin complexes in PEG-6000, Poloxamer-188 and Mannitol were also prepared by fusion and solvent evaporation [14]. Solid dispersion nanoparticles prepared by a supercritical antisolvent process with enhanced bioavailability have also been reported [15]. These approaches deliver improved or optimized pharmacokinetics, yet, similar to the marketed nanoformula they all share the characteristics of complex production method and composition.

In an earlier study we have shown that using a novel, flow chemistry based precipitation method Aprepitant could successfully be formulated with improved, or similar *in vitro* and *in vivo* pharmacokinetic characteristics when compared with the marketed, wet milled nanocrystal formulation, Emend® [16]. In this work our aim was to develop a nanostructured Sirolimus formulation using the same cost-effective production method to overcome the limitations and drawbacks of the industrial standard nanocrystal technology while delivering the improved pharmacokinetic characteristics.

2. Materials and methods

2.1. Materials

Sirolimus was purchased from Leap Labchem Scientific co. Ltd., Hangzhou, China, and Rapamune® tablets (0.5 mg strength, batch number G82845) were purchased in a local pharmacy. SIF powder was purchased from ePhares, Switzerland. FaSSIF and FeSSIF biorelevant media were set up according to the manufacturer's instructions. Methanol was purchased from Molar Chemicals, Budapest, Hungary. All other chemicals were purchased from Sigma.

2.2. Preparation of Sirolimus nanoparticles

The colloid solutions were prepared by precipitation in a batch mode or using a continuous flow precipitation technology (NanGenex Inc., Hungary). In both cases Sirolimus and PVP K90 were dissolved in methanol (solvent) and then water or aqueous solution of SDS (antisolvent) was added. In batch mode the mixing was performed by pipetting the antisolvent to a vial containing the solvent under vigorous stirring (1000 rpm with a 15 × 5 mm magnetic bead in a 25 mm diameter glass vial). In the continuous flow method precipitation of the API from its organic solvent by the addition of an antisolvent takes place as the two liquids are mixed at a high fluid flow velocity [17]. Mixing of the two liquids is faster at high flow rates down to the millisecond range which is required for full mixing [3]. This results in more even precipitation with smaller particles with more uniform particle size [17]. The schematic drawing of the production method in batch mode and in the flow instrument is shown in Fig. 2. The particle size and size distribution of the produced colloid solutions were measured by dynamic light scattering method (DLS, Nanotrac NPA-250, Microtrac Co., USA). The stability of the colloid solutions was also followed by visual observation (crystal forming is visible as the opalescent or translucent colloid solution becomes hazy with visible particles). The colloid was frozen using acetone/dry ice in 100 ml aliquots in 1000 ml flasks and solid formulated by freeze drying using a Scanvac Coolsafe −110 °C instrument (nanostructured Sirolimus).

2.3. Redispersibility and particle size determination

The redispersibility of the samples was monitored by measuring 2–3 mg of sample into glass vials and adding distilled water to 0.25 mg/ml active concentration. The sample was gently hand-shaken for 5 min. The presence of visible particles or the opalescent/translucent (solution) appearance of the sample was followed by visual observation. Samples for particle size measurement tests were taken by diluting the as-synthesized colloid solution to 0.25 mg/ml (for API content) or dispersing a Rapamune® tablet in distilled water at the same active concentration and centrifuging the sample at 2000*g* for 5 min to sediment the non-water soluble, non-colloidal ingredients of the tablet. The particle size of the colloid solutions was determined by dynamic light scattering method