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Nano-amorphous Oral Sirolimus Formulation with Improved Exposure and Low Inter-individual Variability

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1. Introduction

Sirolimus is a macrocyclic lactone which works via the inhibition of the mammalian target of rapamycin (mTOR). It is indicated for the prophylaxis of organ rejection in patients receiving renal and liver transplants [1][2]. Later, the anti-proliferative effect of sirolimus has been shown in various types of cancers and cancer model [3][4][5]. The marketed formulation Rapamune®, which is a wet-milled nanocrystal formula, exhibits low bioavailability, high variability and a moderate food effect. Sirolimus exhibits extensive intestinal and hepatic first-pass metabolism by the CYP3A4 isoenzyme and counter transport by intestinal PgP [6]. These make therapeutic blood monitoring of sirolimus levels necessary for patients. Also, reaching the therapeutic blood sirolimus concentrations in renal cancer patients was found to be challenging when the marketed drug was administered alone, therefore, temsirolimus a prodrug of sirolimus is administered intravenously in these patients [7][8]. The objective of this work was to develop a sirolimus formulation with improved bioavailability which could eliminate the variability and could be an oral alternative to intravenous temsirolimus.

2. Experimental methods

Materials and test items

All API, polymers and other excipients used were pharma grade. Other chemicals were purchased from Sigma. Rapamune® was purchased from a local pharmacy. FaSSIF V_2 and FeSSIF V_2 powders were obtained from Biorelevant (London, UK) and stock solutions were prepared as per the guidance given by the manufacturer.

Preparation of sirolimus nanoparticles

The samples were prepared by precipitation in batch mode. In both cases sirolimus and PVP K90 were dissolved in methanol (solvent) and then water or aqueous solution of sodium dodecyl sulphate (antisolvent) was added. The colloid was solid formulated by freeze drying.

In vitro characterization

Particle size

Before freeze-drying, the particle size of as-synthetized colloid solution was measured by dynamic light scattering (DLS). Rapamune® tablet was dispersed in distilled water and centrifuged, then the particle size of the sample was determined by DLS.

XRD measurement

Crystal structure was investigated by using a Philips PW1050/1870 RTG powder- XRD diffractometer.

Apparent solubility measurement

The crystalline reference, the amorphous sirolimus and the nano-amorphous sirolimus were dispersed in distilled water, FaSSIF (Fasted state simulated biorelevant medium) and FeSSIF (Fed state simulated biorelevant medium). The suspensions or colloid solutions were stirred by magnetic stirring for 24 hours. The suspensions or colloid solutions were filtered and active loading of the filtrates were quantified by HPLC measurement.

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Parallel artifical memebrane permeability assay (PAMPA)

The crystalline reference, Rapamune® tablets and nano-amorphous sirolimus were dispersed in water, FaSSIF or FeSSIF media. The receiver compartment was phosphate buffered saline (pH 7.0) supplemented with 1% Sodium dodecyl sulphate. Incubation time was 4 hours. Concentrations in the receiver compartments were determined by UV spectrophotometry.

Colloid stability measurements in biorelevant media

Nano-amorphous sirolimus was dispersed in water, FaSSIF or FeSSIF media and the colloid stability was detected by nephelometry.

In vivo pharmacokinetic studies in rats

Test articles were prepared by dispersing nano-amorphous sirolimus or Rapamune® tables in water and were administered to Male Wistar rats (220-270 g) orally via esophageal gavage. Before administration rats were fasted overnight. Whole blood was sampled pre-dose, and at 0.5, 1, 2, 3, 4, 6, 8, 24, and 48 hours after administration. Sirolimus concentrations were determined from whole blood samples by a validated LC-MS/MS method.

Clinical PK study

This was a single center, open-label, non-randomized, single dose study in healthy subjects to assess the safety, variability and PK of single ascending doses (0.5-2-10-40 mg) of the nano-amorphous sirolimus formulation, and to assess food effect. As sirolimus stability is reduced by strongly acidic environments, 40 mg famotidine was administered 3 hours before dosing sirolimus in order to increase gastric pH. The 40 mg cohort received a second dose in the fed state. Venous blood samples of were collected and plasma sirolimus levels were quantified by a validated LC/MS/MS method.

3. Results and discussion

In vitro characterization

Nano-amorphous sirolimus produced by nano-precipitation techniqueas-synthesized colloid solution was slightly opalescent with an average particle size (d_{50}) of 30 nm and a d_{90} of 51.5 nm. The average particle size (d_{50}) of the marketed drug was 308 nm (d_{90}=474 nm) an order of magnitude higher than for the nano-amorphous formulation.

The nano-amorphous sirolimus solid formula was stable for 1 year at room temperature. Its structural stability was verified by powder XRD.

The nano-amorphous formulation dispersed in water and in biorelevant media instantaneously and it was stable for 4 hours. The apparent solubility in water and biorelevant media was improved, when compared to Rapamune®. PAMPA permeability for the nano-amorphous formula was significantly better with values 3-4-fold higher when compared to the marketed drug and 5-11-fold higher than the unformulated reference compound.

In vivo pharmacokinetic studies in rats

The in vitro results were in agreement with the in vivo data. The calculated maximal plasma concentration (C_{max}) and exposure (AUC_{inf}) were increased by 3.7-fold and 2-fold, respectively, while t_{max} was reduced by almost 10-fold when compared to Rapamune®**.** Total blood concentration at 24 hours was over 2.8-fold higher for the nano-amorphous formula.

Clinical PK study

The clinical PK results were also in agreement with the pre clinical data. Dose increments resulted in roughly dose proportional increases of C_{max} and $AUC_{0\text{-}48h}$ up to 10 mg.

Mean AUC_{inf} at the 40 mg dose (4300 ng*h/ml) was 28% higher than AUC_{inf} reported following the administration of 90 (2x45) mg Rapamune® when administered alone (3356 ng*h/ml [8]), while it was 11% higher than the AUC reported for the 25 mg intravenous Torisel (3810 ng*h/ml, [8]).

The inter-individual variability of the PK parameters mostly fell in the 20-30 (CV%) range showing that sirolimus was a low-to-moderate variability drug when administered as the nano-amorphous formulation was.

4. Conclusion

the unfavorable PK performance of Rapamune® was improved by the development of a nano-

amorphous formulation, which is therefore a better alternative for oral sirolimus administration. The reduced inter-individual variability might make the therapeutic blood monitoring unnecessary for transplant patients, while increased exposure could allow the treatment of mTOR-responsive malignancies by oral sirolimus instead of intravenous temsirolimus.

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