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Formulation and characterization of a 0.1% rapamycin cream for the treatment of Tuberous Sclerosis Complex-related angiofibromas

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ABSTRACT

Medicines for the treatment of rare diseases frequently do not attract the interest of the pharmaceutical industry, and hospital pharmacists are thus often requested by physicians to prepare personalized medicines. Tuberous Sclerosis Complex (TSC) is a rare disease that causes disfiguring lesions named facial angiofibromas. Various topical formulations of rapamycin (=sirolimus) have been proved effective in treating these changes in small case series. The present study provides for the first time characterization of a 0.1% rapamycin cream formulation presenting good rapamycin solubilisation. The first step of the formulation is solubilisation of rapamycin in Transcutol®, and the second step is the incorporation of the mixture in an oil-in-water cream.

A HPLC stability-indicating method was developed. Rapamycin concentration in the cream was tested by HPLC and confirmed that it remained above 95% of the initial concentration for at least 85 days, without characteristic degradation peaks. The preparation met European Pharmacopoeia microbial specifications throughout storage in aluminum tubes, including when patient use was simulated. Odour, appearance and colour of the preparation were assessed and no change was evidenced during storage. The rheological properties of the cream also remained stable throughout storage.

To conclude, we report preparation of a novel cream formulation presenting satisfactory rapamycin solubilisation for the treatment of TSC cutaneous manifestations, with stability data. The cream is currently being used by our patients. Efficacy and tolerance will be reported later.

1. Introduction

Tuberous Sclerosis Complex (TSC), also known as Bourneville's disease, is a rare genetic autosomal dominant disorder with an estimated frequency of between 1/6000 and 1/10,000 live births (Jacks and Witman, 2015). Angiofibromas develop over time and are very disfiguring, affecting the patient both physically and psychologically.

After observation of the effects of systemic rapamycin on angiofibromas (Bissler et al., 2008; Hofbauer et al., 2008), topical sirolimus was tested on mouse models (Rauktys et al., 2008) and rapidly formulated for humans (Haemel et al., 2010). From then on, several topical forms have been developed using mTOR inhibitors, namely rapamycin and everolimus (Madke, 2013; Balestri et al., 2015).

For the last five years, more than ten formulations have been reported in different pharmaceutical forms (ointment, creams, solutions, etc.) at different concentrations (0.003–1%), from crushed tablets to oral solution, which are not optimal for their tolerance and efficacy. However, all authors described patient improvement, with minimal side effects (except with the solutions), inconsistent percutaneous absorption and systemic diffusion, but recorded recurrence shortly after stopping treatment (Madke, 2013; Balestri et al., 2015; Bouguéon et al., 2015).

We therefore decided to develop a topical treatment and focused our research on three aspects. First, we wanted to offer a formulation containing rapamycin in its solubilized form, in order to obtain immediate bioavailability of the active molecule and thus allowing dose optimization and avoiding the risk of bleeding

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attributed to crushed tablets (Tu et al., 2014). Secondly, we wanted to offer the patient an appealing topical treatment of good appearance in order to improve patient observance and compliance with treatment. Indeed, some patients had reported that ointment was difficult to apply or rough (Park et al., 2014) and sometimes parents decided to reduce the frequency to avoid sending children to school with an oily skin. Thirdly, we wanted to characterize our formulation and assess its stability over time to ensure its efficacy in use by patients.

A cream formulation was therefore developed and a stability-indicating method was then designed and a stability study was performed.

2. Materials and methods

2.1. Materials

Rapamycin powder, polysorbate 80, and sorbitan trioleate were provided by Inresa, (Bartenheim, France). Transcutol® P, olive oil, castor oil, liquid paraffin and sweet almond oil were provided by COOPER (Melun, France). Excipial hydrocrème® was provided by Galderma (Laboratoires Spirig SAS, Toulouse, France). All excipients were of Pharmacopoeia grade.

Methanol (Hipersolv Chromanorm, VWR, Fontenay sous Bois France), was used for HPLC. Water was obtained from a Prima reverse osmosis system (Elga Labwater, Antony, France). All reagents and solvents were of analytical grade.

2.2. Rapamycin solubilisation in various oils and surfactants

Rapamycin powder (10 mg) was incubated with QS 500 mg of oils, solvents or surfactants (Table 1). Mixing was performed under stirring for 60 min at room temperature.

2.3. Cream storage

The cream was packaged in 30 ml aluminum tubes (COOPER, Melun, France) and stored in a climatic chamber conforming to ICH (International Consensus on Harmonization, 2015) at $25 \pm 2\,^{\circ}C$ under $60 \pm 5\%$ relative humidity.

2.4. Sample preparation: rapamycin extraction and recovery

An aliquot of cream (2 g) was introduced into an Erlenmeyer flask with 10 ml of methanol and maintained under stirring for 10 min. All experiments were performed in triplicate. The resulting solution was centrifuged at 900g for 10 min and the supernatant was removed and analyzed for rapamycin content compared to rapamycin in methanol.

2.5. Chromatographic conditions

A high pressure liquid chromatography (HPLC) method was developed, based on Ricciutelli's publication (Ricciutelli et al., 2006). The system was characterized by a Perkin Elmer Series 200 pump, injector and oven. The detector was a diode array detector (Flexar PDA detector, Perkin Elmer, Walthman, USA) operating between 190–700 nm. Chromera software (v4.1.0) (Perkin Elmer, Walthman, USA) was used to quantify the peaks of the chromatograms. The mobile phase consisted of a mixture of methanol and water (80:20 v:v). The flow rate was set at 1 ml/min. A C18 Supelcosil column (150 mm × 4.6 mm, 5 μm) (Supelco® Analytical, Sigma-Aldrich®, Bellefonte, USA) was used and maintained at 50 °C. The sample injection volume was 0.01 ml and the analysis time was 10 min. Rapamycin detection and quantification were processed at 278 nm.

Table 1
Rapamycin solubility in different solvents, oils and surfactants.

oil/surfactant/solvent tested	Rapamycin solubility
Virgin olive oil	<2 mg/ml
Castor oil	<2 mg/ml
Sweet almond oil	<2 mg/ml
Liquid paraffin	<2 mg/ml
Sorbitan trioleate (Span® 85)	<2 mg/ml
Polysorbate 80 (Tween® 80)	<2 mg/ml
Diethylene glycol monoethyl ether P (Transcutol®)	Fully soluble (20.2 mg/ml)

2.6. Method validation

The method was validated according to ICHQ2R1 (International Consensus on Harmonization, 2015).

A standard curve was established with five different rapamycin concentrations in cream: 0.06%, 0.08%, 0.1%, 0.12% and 0.14%. Extraction followed by rapamycin determination was then performed. The linearity of the method was evaluated on three different standard curves.

The repeatability of the method was evaluated by preparing six cream samples concentrated at 0.1% rapamycin on three different days. Each sample underwent rapamycin extraction and determination.

The accuracy of the method was established using three concentration levels (0.08, 0.1 and 0.12%) in triplicate on three different days. Each sample underwent rapamycin extraction and determination.

2.7. Forced degradation

Sensitivity to heat: a 2 g sample of cream was heated at 90 °C for 60 min (n = 3). Then the sample was subjected to the same extraction method as for rapamycin determination in cream. Transcutol® P and Excipial hydrocrème® were also tested alone for heat degradation. No degradation products were observed on chromatograms.

2.8. Physico-chemical stability

Rapamycin determination in the cream was assessed at day 0, 3, 7, 14, 21, 28, 63 and 85 (n = 3 for each day) and mean concentration was expressed as 95% confidence interval of the mean. The mean and confidence interval were considered acceptable if greater than 95% of the initial concentration, without the existence of characteristic degradation peaks.

2.9. Organoleptic appreciation

Odour, appearance and colour of the preparation were assessed at days 0, 3, 7, 14, 21, 28, 63 and 85 (n = 3 for each day). Odour was measured as described in the European Pharmacopoeia, i.e. by spreading 1.5 g of cream on a 6 cm watch glass and smelling the cream after 15 min.

2.10. Rheological measurements

Experiments were performed according to European Pharmacopoeia Monograph 2.2.10. The non-steady flow property of the creams was studied using a Kinexus® rheometer (Malvern Instruments S.A., United Kingdom), with cone plate geometry (diameter 50 mm, angle: 2°) and with controlled shear rates ranging from 0.5 to 50 s^{-1} at room temperature. Two cycles of increasing and decreasing shear rate were performed.