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

Guillaume Bouguéon[a], Frédéric Lagarce[a,b], Ludovic Martin[c], Hélène Pailhoriès[d], Guillaume Bastiat[b], Sandy Vrignaud[a,*]

[a] Pharmacie Centrale, Centre Hospitalier Universitaire d'Angers, Angers Cedex, France
[b] L'Université Nantes Angers Le Mans, INSERM U1066, Micro et nanomédecines biomimétiques, Angers, France
[c] Service de Dermatologie, Centre Hospitalier Universitaire d'Angers, Angers Cedex, France
[d] Laboratoire de Bactériologie, Institut de Biologie en Santé—PBH, Centre Hospitalier Universitaire d'Angers, Angers Cedex, France

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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

* Correspondence to: CHU Angers—Service Pharmacie, 4 rue Larrey, 49933 Angers Cedex 9, France.
E-mail addresses: savrignaud@chu-angers.fr, sandy-vrignaud@hotmail.fr (S. Vrignaud).

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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.

The evolution of viscosity profiles was studied in triplicate directly after the formulation process and after 28 and 84 days incubation at room temperature, and then compared to the cream used as the main ingredient: Excipial Hydrocrème®. Viscosity results were expressed as mean and standard deviation.

2.11. Microbiological study

All studies for microbial contaminations were performed by a certified laboratory following the European Pharmacopoeia 8.0: Monograph 5.1.3: "efficacy of antimicrobial storage for cutaneous preparations" and Monograph 2.6.1: "sterility".

Three conditions were studied (each in triplicate):

- Sample days the same as those for a patient using a medication at home, i.e. opened and removed each day then tested at days 7, 14, 21 and 28.
- Analysis after 28 and 85 days (85 days corresponding to the physicochemical stability of the cream).
- Positive control: *Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (ATCC 9027) were growth on Blood agar plate for 24 h at 37 °C before being suspended for inoculation (105 UFC/g of cream). Cream either containing or not containing rapamycin was artificially infected with *Staphylococcus aureus* or *Pseudomonas aeruginosa* at day 0 and analyzed after 7 days. Infected rapamycin formulations were used as controls to ensure rapamycin did not inhibit microbiological growth. The criterion for evaluation of antimicrobial activity is logarithmic decrease of viable microorganism's number as compared to inoculum value.

To perform analysis, 1 g of cream was diluted in 5 ml of injectable water. Then 0.4 ml was plated on a sheep's blood agar plate (n = 5) (Thermo Fisher Scientific, United Kingdom), and incubated at 37 °C.

According to the European Pharmacopoeia monograph for non-sterile products, the formulation meets microbial requirements if the total aerobic microbial counts are less than 102 cfu/ml or cfu/g, the total combined yeast/mould counts are less than 10^2 cfu/ml or cfu/g and there is no *Staphylococcus aureus* and no *Pseudomonas aeruginosa*.

3. Results and discussion

The absence of commercially available medicines for the treatment of main rare disease is likely to lead to the development of a preparation in a hospital pharmacy. Designing a suitable cream for patients includes the choice of an appealing formulation, study of the drug's physicochemical stability in the cream, and ensuring satisfactory microbial quality.

3.1. Solubility of rapamycin in various oils, solvents and surfactants

Facial angiofibromas appear during infancy, between 2 and 5 years of age, and grow progressively up to teenage years. In view of children's and adolescents' acne-prone skin, an oil-in-water emulsion basis appeared to be the best compromise for TSC patient treatment. However, rapamycin is poorly soluble in water. The first step in making a compound was therefore the solubilization of rapamycin in an appropriate solvent prior to topic formulation. Solubilization of the drug in the formulation is important both to allow its diffusion through the skin and also for ease of application of the cream. Different ingredients were selected to prepare a safe formulation for topical application (listed in Table 1).

Table 2
Composition of rapamycin cream.

Component	Quantity
Rapamycin	0.03 g
Transcutol®	1.5 g
Excipial Hydrocrème®	QS 30 g

Different reports indicate that rapamycin is easily incorporated in liquid paraffin prior to topical formulation (Madke, 2013; Balestri et al., 2015). We therefore first tested rapamycin solubility in liquid paraffin, and observed less than 2 mg/ml solubility.

We then assessed different safe ingredients usable for topical application. Table 1 shows rapamycin solubility in three ingredients. The best candidate was Transcutol® that solubilizes rapamycin extemporaneously, whereas the drug was only slightly soluble in other ingredients. Transcutol®, i.e. diethylene glycol monoethyl ether, is already used in marketed medicines. Furthermore, Transcutol® has been shown to be a drug penetration enhancer in the skin (Mura et al., 2000). This property appeared very interesting to exploit, as it could improve treatment of angiofibromas. The rest of the study was therefore performed with Transcutol®.

Because Rapamycin is solubilized in a solvent which is then incorporated in an emulsion (the cream excipient), its level of solubility should be higher than 1% (i.e 10 mg/ml) in order to obtain a final concentration of 0.1% active drug while adding less than 10% of solvent in the cream, which is important to maintain the stability of a cream which is an emulsion. The solubility of rapamycin in Transcutol® allowed use of only 5% of this excipient in the final formulation as explained below.

3.2. Formulation of rapamycin cream

The cream was prepared by first solubilizing rapamycin in Transcutol®. This mixture was then progressively added to Excipial Hydrocrème® under manual stirring for several minutes. The formulation is presented in Table 2. The rapamycin dosage in the cream was 0.1% (w/w), which is in the low range of the previously published formulations but here the active drug was solubilized and a much higher level of activity was therefore expected. As explained above, the high level of solubility of rapamycin in Transcutol® would allow a dosage of 0.4% if needed without affecting the appearance of the cream or physical stability.

Because of rapamycin toxicity, preparation was performed in a low pressurized glove-box to protect the pharmacy technician. Excipial Hydrocrème® was selected from various commercially available formulations as it is an oil-in-water emulsion composed of safe ingredients, is non-comedogenic and not unpleasant to use. The final cream has a slight oil odour, a homogeneous appearance and is white in colour. These characteristics remained constant throughout the study.

3.3. Stability study

Once the cream had been prepared, a stability study was performed. The physico-chemical stability study of rapamycin requires drug extraction and HPLC method validation to determine rapamycin concentration. Drug recovery after rapamycin extraction by methanol from the cream was 64.2% ± 1.2%. This method did not detect the totality of rapamycin in the cream. Nevertheless, such extraction was repeatable and allowed detection of degradation products, as shown in Fig. 1b.

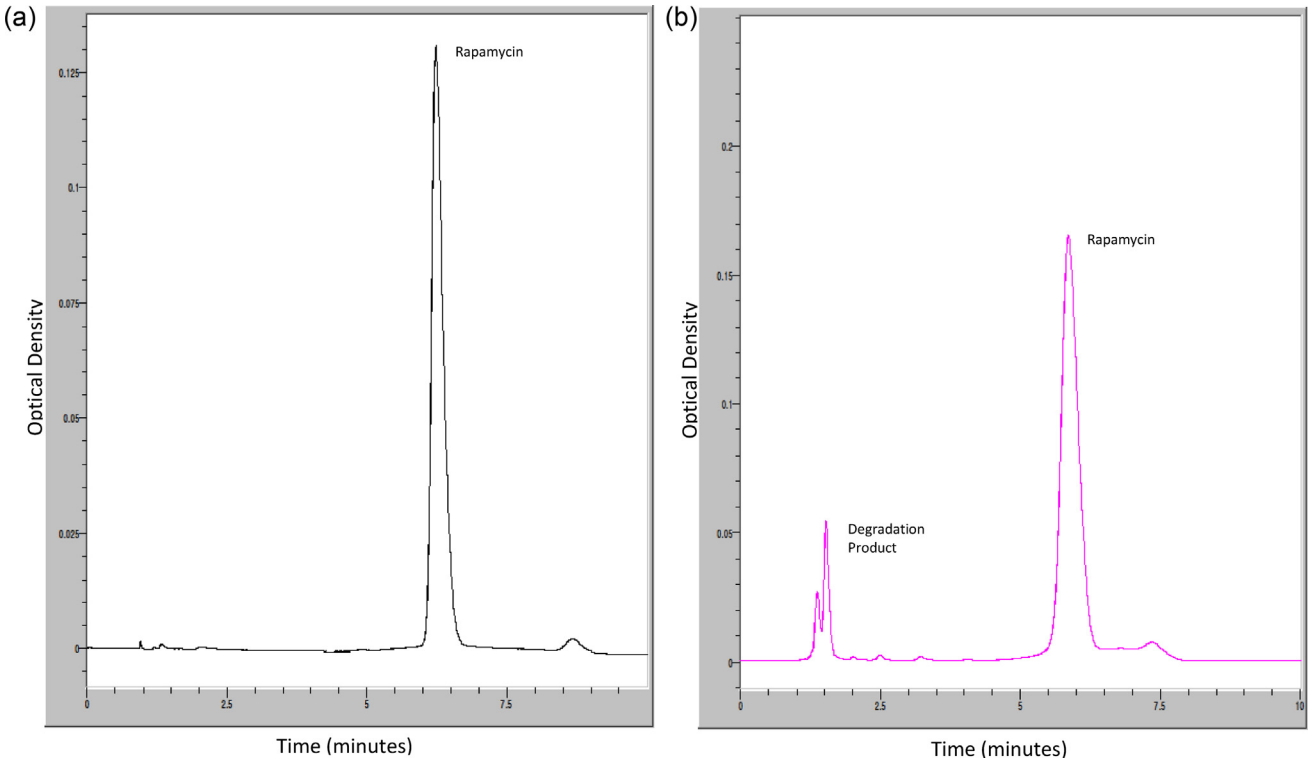

Fig. 1. Examples of rapamycin chromatogram: (a) reference cream 0.1%, (b) forced degradation by heat.

A well-defined and symmetric peak of rapamycin was obtained using the HPLC method (Fig. 1a). The correlation coefficient, ($r^2 = 0.994$) indicated the linearity in the interval range of [0.06–0.14%]. Forced degradation was obtained by heating (Fig. 1b) method, and permitted validation of the method as indicating stability, as it allowed detection of degradation products at retention times between 1 and 2 min. Heat was chosen as a non-specific degradation process as many degradation pathways follow Arrhenius' law and are thus governed by temperature.

Repeatability, assessed at 18 determinations of the test concentration (0.1%) (6 replicates/3 different days), was systematically lower than a coefficient of variation of 7%. Accuracy, assessed at three concentrations (0.08–0.1 and 0.12%), on three days (9 determinations), was systematically higher than 95% of the expected value. Rapamycin was eluted in 6 min, as shown in Fig. 1a.

The evolution of the rapamycin concentration in the preparation is indicated in Table 3. The mean percentage and the 95% confidence interval around the mean were systematically higher than 95% of the initial concentration, indicating that rapamycin was stable in the cream. Furthermore, no degradation products were detected on chromatograms throughout the study.

3.4. Rheological properties of the cream

The viscosity of initial and formulated creams decreased when the shear rate increased and vice versa, showing the rheofluidifiant properties of the formulations (Fig. 2). In addition, the low standard deviations for the 3 independent formulated creams proved the repeatability of the formulation process. Finally, the flow profiles over time for the 3 independent formulated creams remained unchanged, showing the time stability of the formulated creams (Fig. 3). Wilcoxon (for Fig. 2 data) and Kruskal-Wallis (for Fig. 3 data) statistical tests did not show any significant difference regarding the formulation nature, the time and shear rate ramp. Such properties are satisfactory for patient use.

3.5. Microbial stability study

The rapamycin cream remained within European Pharmacopoeia specifications for contamination throughout study after 28 and 85 days when stored at 30 °C (Table 4). No *Staphylococcus aureus* or *Pseudomonas aeruginosa* were detected in any case. A positive control was performed with artificially *Pseudomonas aeruginosa* or *Staphylococcus aureus*-ontaminated cream. Microbial growth was observed, demonstrating that rapamycin did not inhibit growth. After 28 days incubation, no increase of viable microorganisms was observed as compared to day 2.

We also tested conservation mimicking patient use, i.e. the tube was opened every day for one month. Microbial contamination assessed at days 7-14-21 and 28 was also satisfactory (below European Pharmacopoeia limits, Table 5). This result was attributed to (i) triclosan, disodium EDTA and chlorhexidine

Table 3
Sirolimus concentration in the cream expressed as 95% confidence interval (CI) (n = 3) relative to concentration on day 0.

Time (days)	3	7	14	21	28	63	85
CI95 (% compared to day 0)	101.5–102.5	102.8–103.2	103.9–104.1	106.7–107.3	105.5–106.5	99.7–100.3	100.4–101.6

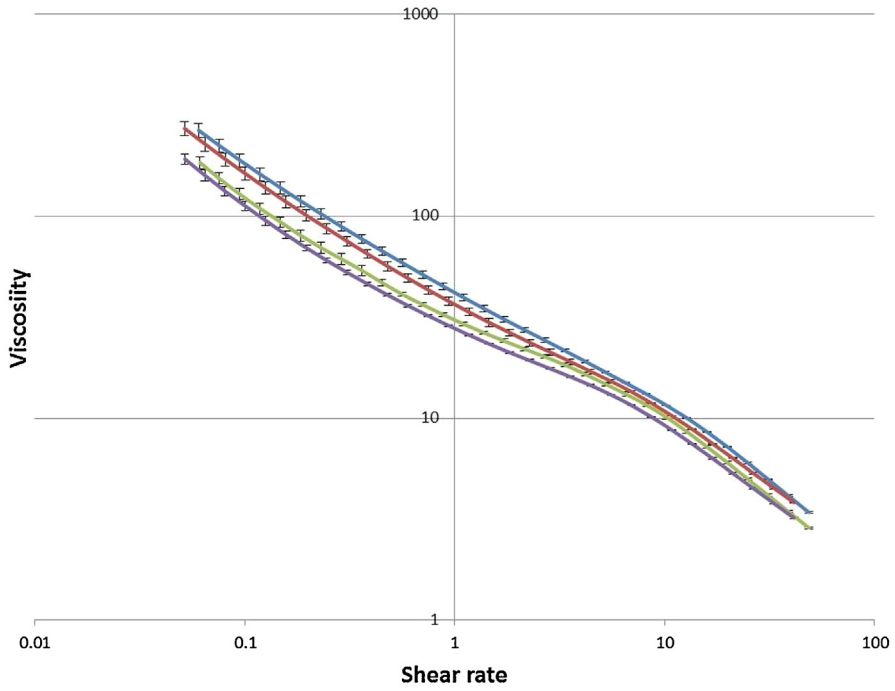

Fig. 2. Rheological comparison between Excipial Hydrocrème® and cream containing rapamycin, at day 0.
Legend: blue line: Excipial Hydrocrème® shear rate increase, red line: Excipial Hydrocrème® shear rate decrease, green line: rapamycin cream shear rate increase, purple line: rapamycin cream shear rate decrease. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

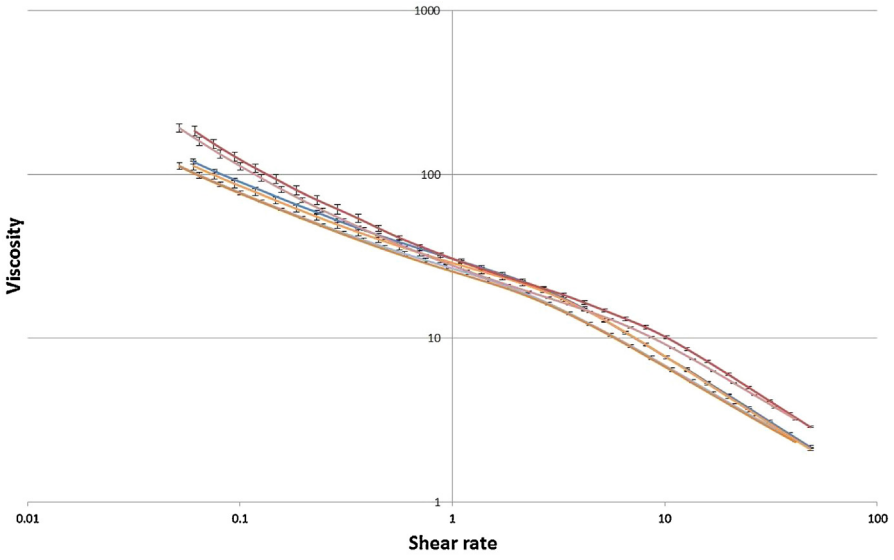

Fig. 3. Rheological properties of cream containing rapamycin over time.
Legend: Day 0: Dark purple line: shear rate increase. Light purple line: shear rate decrease. Day 28: Dark orange line: shear rate increase. Light orange line: shear rate decrease. Day 85: Dark blue line: shear rate increase. Light blue line: shear rate decrease.

Table 4
Microbial contamination of cream during storage. CFU/g = Colony forming units/gram of cream. Expressed as average of 3 replicates for each day.

Day	0	28	85
Number of CFU/g	16.7 ± 11.8	1 ± 1.4	0 ± 0
Pseudomonas aeruginosa	none	none	none
Staphylococcus aureus	none	none	none

Table 5
Microbial contamination of cream in patient use condition. UFC/g = Colony forming units/gram of cream. Expressed as average of 3 replicates for each day.

Day	7	14	21	28
Number of CFU/g	4.2 ± 3.1	1 ± 1.4	0 ± 0	1 ± 1.4
Pseudomonas aeruginosa	none	none	none	none
Staphylococcus aureus	none	none	none	none

dihydrochloride, which are preservatives present in Excipial Hydrocrème® and (ii) appropriate packaging in aluminum tubes, limiting contact with contamination.

4. Conclusion

We present for the first time a formulation with solubilized rapamycin. To date, publications have only reported formulations made from crushed tablets or oral solutions of rapamycin, which have presented major drawbacks: (i) when using tablets the drug is partially or even not solubilized in the topical formulation, and it is thus less (or even not) bioavailable to exert its effect. Furthermore, skin damage has been reported due to inadequate crushing of tablets. (ii) When using oral formulations, oral ingredients are present in the topical composition and are likely to cause side-effects.

Incorporation of rapamycin and homogeneous distribution in the topical formulation are more effective if the rapamycin is previously solubilized in a solvent. Moreover, skin diffusion and drug bioavailability will be enhanced. Our best results were obtained with Transcutol® (highly purified diethylene glycol monoethyl ether) commonly used as an ingredient in topical formulations. Transcutol® is also an excellent permeation agent that enhances drug diffusion through the skin (Mura et al., 2000). Absolute galenic control of our preparation will permit the best drug distribution, will increase bioavailability and efficiency, will reduce the necessary concentration of rapamycin and its cost.

We report here for the first time a stability study of a topical formulation containing rapamycin. The physico-chemical stability of the cream was 85 days. No degradation products were detected during this time. Microbiological stability studies showed that patients can use the cream without risk of infection. Viscosity measurements are recommended in the European pharmacopoeia. The experiments revealed that incorporating rapamycin and Transcutol® in the cream did not change its rheological properties. Furthermore, the organoleptic characteristics (odour, appearance and colour) were unaffected.

Such a preparation warrants clinical evaluation, with the aims of optimizing dosage and proposing a long term maintenance scheme to avoid recurrence of cutaneous manifestations of TSC. The 0.1% rapamycin cream has been used for several months by TSC patients in our hospital. The efficacy and tolerance will be reported later.

Conflict of interest

The authors confirm they have no conflicts of interest with regard to the content of this article.

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References

Balestri, R., Neri, I., Patrizi, A., Angileri, L., Ricci, L., Magnano, M., 2015. Analysis of current data on the use of topical rapamycin in the treatment of facial angiofibromas in tuberous sclerosis complex. J. Eur. Acad. Dermatol. Venereol. 29 (1), 14–20.

Bissler, J.J., McCormack, F.X., Young, L.R., Elwing, J.M., Chuck, G., Leonard, J.M., et al., 2008. Sirolimus for angiomyolipoma in tuberous sclerosis complex or lymphangioleiomyomatosis. N. Engl. J. Med. 358, 140–151.

Bouguéon, G., Vrignaud, S., Martin, L., Lagarce, F., 2015. Traitement des angiofibromes de la sclérose tubéreuse de Bourneville par des préparations topiques à base de sirolimus: état des lieux de la préparation en France et revue de la littérature. Ann. Dermatol. Venereol. 142 (12), S668–S669.

Haemel, A.K., O'Brian, A.L., Teng, J.M., 2010. Topical rapamycin a novel approach to facial angiofibromas in tuberous sclerosis. Arch. Dermatol. 146 (7), 715–718.

Hofbauer, G.F.L., Marcollo-Pini, A., Corsica, A., Kistler, A.D., French, L.E., Wuthrich, R. P., Serra, A.L., 2008. The mTOR inhibitor rapamycin significantly improves facial angiofibroma lesions in a patient with tuberous sclerosis. Br. J. Dermatol. 159 (2), 473–475.

International Conference on Harmonisation. Q2 (R1): Validation of Analytical Procedures: Text and Methodology, 2015, 6–12.

Jacks, S.K., Witman, P.M., 2015. Tuberous sclerosis complex: an update for dermatologists. Pediatr. Dermatol. 17 doi:http://dx.doi.org/10.1111/pde.12567.

Madke, B., 2013. Topical rapamycin (sirolimus) for facial angiofibromas. Indian Dermatol. Online J. 4 (1), 54–57.

Mura, P., Faucci, M.T., Bramanti, G., Corti, P., 2000. Evaluation of transcutol as a clonazepam transdermal permeation enhancer from hydrophilic gel formulations. Eur. J. Pharm. Sci. 9, 365–372.

Park, J., Yun, S.K., Cho, Y.S., Song, K.H., Kim, H.U., 2014. Treatment of angiofibromas in tuberous sclerosis complex: the effect of topical rapamycin and concomitant laser therapy. Dermatology 228, 37–41.

Rauktys, A., Lee, N., Lee, L., Dabora, S.L., 2008. Topical rapamycin inhibits tuberous sclerosis tumor growth in a nude mouse model. BMC Dermatol. 8 (1), 1–9.

Ricciutelli, M., Di Martino, P., Barboni, L., Martelli, S., 2006. Evaluation of rapamycin chemical stability in volatile-organic solvents by HPLC. J. Pharm. Biomed. Anal. 41 (3), 1070–1074.

Tu, J., Foster, R.S., Bint, L.J., Halbert, A.R., 2014. Topical rapamycin for angiofibromas in paediatric patients with tuberous sclerosis: follow up of a pilot study and promising future directions. Australas. J. Dermatol. 55, 63–69.