Long-term stability of 0.1% rapamycin hydrophilic gel in the treatment of facial angiofibromas

Guillaume Le Guyader, Victoire Vieillard, Karine Andrieux, Mylène Rollo, Olivier Thirion, Pierre Wolkenstein, Muriel Paul

Long-term stability of 0.1% rapamycin hydrophilic gel in the treatment of facial angiofibromas

Guillaume Le Guyader[1], Victoire Vieillard[1], Karine Andrieux[2], M. Rollo[1], Olivier Thirion[1], Pierre Wolkenstein[3], Muriel Paul[1]

[1] Pharmacy Department, Henri Mondor Hospital Group, AP-HP, 51 avenue du Maréchal de Lattre de Tassigny, 94010 Créteil, France.
[2] Unité de Technologies Chimiques et Biologiques pour la Santé (UTCBS), UMR CNRS 8258 U1022 INSERM, Université Paris Descartes, 12 Rue de l'École de Médecine, 75006 Paris, France.
[3] Department of Dermatology, Henri Mondor Hospital Group, AP-HP, 51 avenue du Maréchal de Lattre de Tassigny, 94010 Créteil, France.

[1] **Corresponding author:** Guillaume Le Guyader, Pharm D, Pharmacy Department, Henri Mondor Hospital Group, AP-HP, 51 avenue du Maréchal de Lattre de Tassigny, 94010 Créteil, France ; Tel : 0149814753 ; email : guillaume.leguyader@aphp.fr.

ABSTRACT

Objectives : In recent years, various formulations containing rapamycin were tested, mainly with petrolatum to treat facial angiofibromas in tuberous sclerosis, especially in children. Nevertheless, a weak tolerance was found due to irritations and bleeding. Moreover, efficacy was insufficient in young adults. The aims of this study were to develop and characterize a hydro-alcoholic gel containing solubilized rapamycin, to realize the stability study at 4°C up to one year. **Methods :** Two different gels at 0.1% rapamycin were performed with or without α-tocopherol and urea. Different methods were used to characterize the gels: HPLC, gas chromatography, pH, visual observation and optical microscopy. A physicochemical and microbiological stability study was also conducted during 1 year at 4°C. **Results :** Whatever the formulation, gels were physicochemically and microbiologically stable up to 1 year at 4°C: Organoleptic characteristics and pH were unchanged, no significant decrease was found in rapamycin concentration, tocopherol droplets size was constant and the rheological comportment unmodified. **Conclusions :** The present study provides, for the first time, extended stability data concerning a hydro-alcoholic gel of rapamycin.

Key words: rapamycin, topical formulation, tuberous sclerosis, stability.

INTRODUCTION

Tuberous sclerosis complex (TSC), also known as Bourneville's disease, is an autosomal dominant disorder, mainly characterized by the occurrence of multiple hamartomas that can affect different organs. It preferentially reaches the skin (hypomelanic spots, angiofibromas, Koenen tumors), brain (cortical tubers, subependymal nodules) and kidney (renal angiomyolipoma and cysts) while the eyes (retinal hamartomas), heart (rhabdomyomas) and lungs are less frequently affected [1].

Penetrance and expressivity are extremely variable, which explains the high variability of clinical symptoms ranging from non-severe forms (e.g. limited to the skin), to more severe forms. The frequency is estimated between 1/6,000 and 1/10,000 live birth and a population prevalence of around 1/20,000 [2], but it is probably underestimated because patients come to consultation only for the severe forms.

Among the different cutaneous lesions associated with TSC, facial angiofibromas are one of the most commonly cutaneous manifestations. They can be highly disfiguring lesions and can have a significant impact on patient quality of life [3]. They appear as red or brownish papules on the center part of the face, on the cheeks and chin [4]. TSC results from the over-activation of the mammalian target of rapamycin (mTOR) in dermal cells. These cells then produce an epidermal growth factor, epiregulin, which also stimulates the proliferation of fibroblasts and keratinocytes in the dermis and the angiogenesis; these proliferation are responsible for the formation and progression of angiofibromas [5,6].

Many symptomatic treatments have been developed to reduce the appearance of angiofibromas such as cryosurgery, dermabrasion, curettage, excision or laser ablation. But some of these are expensive and the results are disappointing with limited efficacy and frequent recurrences and should require regular sessions [7,8]. Recently, based upon the pathophysiology of the disease, the interest of mTOR inhibitors as rapamycin (sirolimus) or everolimus and of calcineurin inhibitors as tacrolimus has been studied. In order to limit systemic side effects due to oral administration, different topical formulations were tested with the three drugs. Wataya-Kaneda et al. [9] have shown that no improvement was observed with Protopic® alone (tacrolimus ointment 0.03%, Astellas Pharma Europe), while an association with rapamycin resulted in significant

reduction of angiofibromas, probably due to the fact that tacrolimus is not a direct mTOR inhibitor. Everolimus was also tested and a likely efficacy was reported [10]. Rapamycin was the most studied mTOR inhibitor over the past ten years, used as oral solution (Self Micro-Emulsifying Drug Delivery System (SMEDDS)) or as ointment with crushed tablets in petrolatum on skin. Regardless of the blood level, efficacy and safety were highlighted [11,12]. However, Rapamune® oral solution 1 mg/ml was not suitable for topical administration in terms of tolerance, causing irritation and burning sensations [13] probably due to high levels in surfactant agents. Moreover, as rapamycin is insoluble in petrolatum or in Dexeryl®, the cutaneous bioavailability of these preparations is expected to be low, thereby explaining a certain delay to reach a clinical response (several months) and the lack of total disappearance of angiofibromas in the young adults. Regarding young children, good efficacy was observed along with maximal clearance [12,13] probably due to their skin characteristics, i.e. thinner and more permeable. As to ointment with crushed tablets in petrolatum, the presence of solid fragments may be responsible for the risk of bleeding and also, uncomfortable sensation of oily skin was observed. Recently, Bouguéon et al. [14] proposed a cream with 0.1% rapamycin solubilized in 5% Transcutol® to avoid any bleeding issue and this oily sensation, with stability data.

As a result, this work aimed at developing and characterizing a hydro-alcoholic gel containing solubilized rapamycin and free of surfactant so as to improve the active molecule bioavailability, reach a good appearance and allow cutaneous tolerance. Efficacy was tested on young adults having not received any systemic treatment. Finally, a long-term stability study at 4°C was carried out to determine its shelf life.

METHODS

Gel preparation

Two formulations were developed. Carbopol 974P® (Duchefa Farma B.V., Haarlem, Nederland) was used as gelling agent. Rapamycin (Inresa, Dholka, Ahmedabad, India) was solubilized in ethanol (VWR, Fontenay-sous-Bois, France). Glycerol (Sigma-Aldrich, St. Quentin Fallavier, France) was added with or without α-tocophérol (Sigma-Aldrich, St. Quentin Fallavier, France) and urea (Sigma-Aldrich, St. Quentin Fallavier, France) at room temperature. Twenty grams of gel were then packaged in aluminum tubes (Cooper, Melun, France) and stored in a controlled cold area (5±3°C) for all the duration of the study.

Chromatographic conditions

A validated method was used for this study based on the publication of Yuri V. Il'ichev's et al. [15]. Samples were analyzed using an Ultimate 3000 coupled with a Photodiode Array Detector PDA-3000 (Thermo Fisher Scientific, Villebon-sur-Yvette, Courtaboeuf, France) operating between 190 to 800 nm. Separation was achieved using a 250 x 4.6 mm Vintage Series KR C18 5 µm column maintained at 40°C (Interchim, Montluçon, France). A 10 x 4.0 mm guard cartridge modulo-cart Interchim QS 2UM was used. The mobile phase A consisted of mixture of water-formic acid 0.003 vol% (Merck, Suprapur®, Darmstadt, Germany) and mobile phase B was acetonitrile-formic acid 0.003 vol%. Acetonitrile and methanol (Chromasolv®) were HPLC-grade (Sigma-Aldrich, St. Quentin Fallavier, France). The gradient program used was an elution gradient of 50:50 (phase B:phase A) to 90:10 (phase B:phase A) over 25 minutes. The flow rate was 1.0 ml/min and the injection volume was 50 µL. Rapamycin detection and quantification was processed at 277 nm for an acquisition time of 40 min. The data were processed using Chromeleon® software (v 6.8).

Method validation

The RP-HPLC method was validated according to the International Consensus on Harmonization guidelines (ICHQ2R1). A stock solution of rapamycin was prepared by dissolving 10mg of rapamycin in a final volume of 10ml of methanol. Then, working standard solutions were prepared by serially dilution with methanol from the above stock solution to obtain five different rapamycin concentrations: 6, 8, 10, 12 and 14 µg/ml. The linearity of the method was evaluated on three different standard curves on different days. Accuracy was determined by calculating the percentage of recovery. Precision of the method was assessed by measuring intra (repeatability) and inter-day (intermediate precision) variation, both of which were expressed as relative standard deviation (RSD). Repeatability was evaluated by the repeated analysis (6 injections) of a solution containing 10 µg/ml of rapamycin. Inter-day variation was determined by three replicate on three different days of each point of the range of the drug.

Rapamycin extraction and recovery

To appreciate the extraction coefficient, five different rapamycin concentrations in gel was prepared in triplicate (0.06%, 0.08%, 0.1%, 0.12% and 0.14%) and measured by HPLC. For this, 0.1 g of the preparation was weighed and poured in a volumetric flask (10 ml). Methanol was added and the flask was placed in ultrasonic bath for 10 minutes. The resulting solution was centrifuged in a Sigma centrifuge 1-14 K at 10 000 rpm for 5 minutes and the supernatant was removed and analyzed by HPLC. This solution was compared to rapamycin methanol stock solution at the same concentration.

Forced degradation

Stress studies were performed to demonstrate specificity of the stability-indicating method. To identify one major degradants of rapamycin, i.e. secorapamycin, a standard

solution at 10 µg/ml of secorapamycin, obtained from Cayman Chemical Company (Michigan, USA) and diluted in methanol, was subjected to the HPLC method.

For each formulation, 0.1 g of gel (n=3) was subjected to stress condition of acid (0.1 N HCl; heated for 1 h at 40°C), base (0.01 N NaOH; heated for 1 h at 40°C), and oxidation (H_2O_2 1 vol; heated at 40°C for 1, 2, 3, 6 and 24 h, white light). Then, an extraction was performed under the same conditions as presented above and analyzed by HPLC. Blank gel was also tested alone.

Organoleptic characteristics/pH

The different organoleptic characteristics evaluated were:

- Appearance: An aliquot of gel was placed on a microscope slide and was inspected visually for their colour, homogeneity (presence of any aggregates) and grittiness (presence of particles or grits). The gel was smelled to evaluate the presence of alcohol odour.
- Consistency: The stickiness was evaluated by spreading an aliquot of blank gel (without rapamycin) on the skin.
- Size and repartition of α-tocophérol droplets and detection of possible rapamycin crystals: Microscopic observation (microscope Olympus®IM coupled to a Sony camera XCD-U100CR) was performed to ensure the homogeneity of the gel by searching the absence of aggregates and following the evolution of the size of lipid droplets (α-tocophérol). Particles and lipid globules were observed using the Archimed® software (Microvision Instruments) and measuring the mean diameter and the standard deviation of lipid droplets with Ellix™ software (Microvision Instruments). This analysis

was performed on about 100 units for each formula. The diameter, width and surface of lipid droplets were evaluated.

- pH determination: it was determined before each analysis using a calibrated pH meter Consort P901 (Belgium).

Determination of residual ethanol by gas chromatography (GC)

Residual ethanol was analyzed using Ultra Trace GC gas chromatography system (Thermo Electron Corporation, Milan, Italy). The column was an Interchim 30 m x 0.53 mm UB-624 column 3 µm. A solution of acetonitrile 1 g/L (MeCN) was used as the internal standard. The detector was a Flame Ionization Detector (FID). The injector port temperature was 140°C with a split ratio 1:5 and the detector temperature was 240°C. Column temperature was maintained at 40°C for 10 min. The flow rate of carrier gas is 6 ml/min. For the determination of residual ethanol, an aliquot of gel (50 mg) was weighed and poured with water and diluted in a volumetric flask to a final volume of 20 ml. Then, 1 µL of 50:50 (Sample:MeCN) was injected. The residual ethanol was expressed in percentage weight/volume (g/100 ml).

Rheological measurements

Rheological measurements were performed on an Anton Paar MCR-102 (Modular Compact Rheometer, Graz, Austria) equipped with plate-plate geometry of 100 mm diameter and Peltier temperature control device with thermostatic hood. Viscosity was measured as a function of the shear rate for $d\gamma/dt$ between 0.1 and 100 s^{-1} at 20.0±0.1°C. Experiments were run in triplicates directly after the formulation process (day 0) and after 30 and 365 days after storage at 4°C. Viscosity results were expressed as mean ± SD.

Stability study

Samples (0.5 g) were withdrawn from tubes stored at 5±3°C, at different times (day): 0, 2, 7, 14, 30, 65, 100 and 365.

Different methods have been used to characterize both formulations, evaluate their microbial quality and study the drug physicochemical stability before their preliminary clinical evaluation. For each time, were evaluated the organoleptic characteristics and pH, the percentage of rapamycin remaining, the residual ethanol concentration. The rheological properties were evaluated at day 0, 30 and 365 and the microbiological cleanliness was evaluated at day 0 and day 365.

The expiry date (days), based on a limit of 5% degradation, was calculated from the determination of T95% as part of the process of assigning product shelf life in accordance with the recommendations of ICH.

Microbiological stability

Microbial contamination of formulations has been tested in accordance with the European Pharmacopoeia. 1 g sample to be tested freshly manufactured or already stored for 1 year at 2-8°C were diluted to 1/50 in meat peptone neutral solution. 5 ml of solution was filtered at a rate of 1 filter per 1 ml. Thereafter, the filters were successively rinsed with 50 ml sodium chloride solution and with 50 ml meat peptone neutral solution to remove residual ethanol.

After contact of a filter membrane with soybean casein digest agar, the plates are incubated at 32°C and total aerobic microbial count (TAMC) is determined after a 3-day storage. The use of Sabouraud dextrose agar incubated at 25°C for 5 days allowed to determine the total combined yeasts/moulds count (TYMC). Results were expressed as a colony forming unit per gram (CFU/g) against specification limits ($<10^2$ CFU/g for TAMC and <10 CFU/g for TYMC).

Statistical analysis

All values were expressed as mean ± standard deviation (SD). Data were compared using one-way ANOVA. In all cases, a difference was considered significant at $p<0.05$.

RESULTS

Characterization of the formulations

The characterization of the formulations was evaluated in five batches for formula 1 (n=5) and four batches for formula 2 (n=4).

Physicochemical characteristics of rapamycin gels :

Organoleptic characteristics and pH of the different gels are summarized in Table 1.

Table 1 : Organoleptic characteristics and pH values for each gel formulation.

Organoleptic characteristics	**Formula 1**	**Formula 2**
Appearance	Smooth and homogeneous	
Colour	Transparent	Slightly opalescent
Stickiness	No sticky	
Odour	Slight alcohol odor	
Size of α-tocophérol drops	Not applicable	8.7 ± 3.8 µm
Rapamycin crystals	Not observed	
pH value	6.60 ± 0.07	6.70 ± 0.10

Both formulae appeared as smooth, homogeneous and no sticky gels with a slight alcohol odor and a pH value around 6.6-6.7, compatible with skin application. A slight opalescence was observed in formula 2 due to tocopherol droplets with a size of 8.7 ± 3.8 µm. Moreover, microscopy observations have evidenced the absence of rapamycin crystals in both formulations confirming the dissolution of drug into these gels.

Concerning rheological measurements, both formulations exhibited a rheofluidifiant character. Indeed, the initial viscosity decreased when the shear rate increased. There is no difference between formula 1 and formula 2. The viscosity of the gels was closed to 20 Pa.s that is compatible for a skin application.

Determination of residual ethanol by gas chromatography :

The chromatogram obtained by GC, showed that ethanol and acetonitrile, were eluted at 2.40 min and at 2.96 min, respectively. The ethanol concentrations (g/100ml) were of 18.5 ± 1.5 % for formula 1 and 18.7 ± 2.79 % for formula 2, respectively. A 33% loss after manufacturing was observed with respect to the theoretical concentration.

Rapamycin content in gels :

Validation of the HPLC method to measure rapamycin content in gel :

The determination of drug content in formulations and their stability study required a validation of the HPLC method used to measure rapamycin concentration. This method permitted to obtain a well-defined peak and a good resolution to ensure a good separation between isomers. As shown in Figure 1, the chromatogram showed a well-defined and symmetrical main peak (asymmetry factor=1) of the rapamycin beta (β) isomer and also one of the drug's gamma (γ) isomer, with retention times (rt) of approximately 20 min and 21 min, respectively. Linear response signal *versus* concentration was obtained over the concentration range of 6-14 µg/ml, with a correlation coefficient of r^2=0.998. The mean recovery rate was 100.03 ± 1.25% confirming the accuracy of the method. The relative standard deviations (RSD) were 1.26% and 1.99% for the repeatability and the intermediate precision, respectively, which were satisfactory values.

Rapamycin extraction and recovery :

The extraction in methanol from the gel, allowed a good recovery of 92.2 ± 0.06% for formula 1 and 94.6 ± 0.05% for formula 2. Then rapamycin assay in the gel was determined from the standard curve using rapamycin standard dissolved in methanol.

Rapamycin concentration :

Using the validated HPLC method, the drug content was determined in gels: 0.098 ± 0.003% for formula 1 and 0.090 ± 0.005% for formula 2 which were closed to the expected concentration of 0.1%.

Forced degradation :

Forced degradation of gel was performed in order to verify the ability of the HPLC method to measure the presence of rapamycin and its degradation products. No interfering peak was observed regardless of the stressed condition applied, suggesting the specificity of the method and its suitability for the routine quality control analyses. Adequate resolution between the peaks relevant to rapamycin and rapamycin degradation products was also observed. Moreover, the addition of tocopherol in formula 2 appears to protect rapamycin from oxidative stress confirming the interest to add this antioxidant ingredient (Figure 2).

Long-term stability study of rapamycin gels

Physicochemical characteristics :

The organoleptic characteristics (appearance, modification of colour, stickiness) of the gels remained unchanged throughout the period tested. No particle of rapamycin was detected by microscopic examination. Moreover, no modification of rheological character of gels was observed as a function of time (Figure 3).

The α-tocopherol droplets size was similar with a diameter ranging from 6.6 ± 3.25 µm to 9.6 ± 1.63 µm.

Regarding pH, no significant change after one year of storage was observed, pH were 6.7 ± 0.10 (p= 0.13) for formula 1 (n=3) and 6.7 ± 0.08 (p= 0.71) for formula 2 (n=3).

Determination of residual ethanol by gas chromatography (GC) :

Throughout the storage period, residual ethanol did not significantly alter for the two formulae (p=0.92 and p=0.40 for formula 1 and formula 2, respectively). The solvent

fell within 20.0 ± 2.21 % (g/100 ml) for formula 1 (n=5) and 20.5 ± 2.46 % for formula 2 (n=4) as originally found.

Percentage of rapamycin remaining :

Regarding rapamycin concentration, no significant change occurred after one-year storage (p=0.17 for formula 1 and p=0.12 for formula 2). The concentration levels were 99.4 ± 2.23 % and 101.0 ± 1.94 % for formula 1 (n=5) and formula 2 (n=4), respectively. Due to these results, the T95% was not calculated because never reached evidencing that the gels can be stored more than one year without any loss of drug. As shown in Figure 4, the areas under curve (AUC) and the two chromatograms at days 0 and 365 for formula 2 were comparable. The same result was observed with formula 1. Moreover, the degradation products, notably secorapamycin, were undetected.

Microbiological stability :

All preparations were conform to the current European Pharmacopoeia 8.0 and showed that the TAMC and the TYMC were less than 10 colonies per g (CFU/g) in the storage conditions at 4°C. In addition, there were no suspected colonies of Staphylococcus aureus and Pseudomonas aeruginosa found in the selective media of any samples. Moreover, the same result was observed after 1 year of storage.

DISCUSSION

The results showed that our hydro-alcoholic gel containing 0.1% of rapamycin and formulated with or without α-tocopherol and urea, was stable at least for one year at +4°C and protected from light. No degradation products are detected and there was no change in the physico-chemical or microbiological characteristics of the gel. The gel had a smooth and homogeneous texture with no trace of rapamycin precipitate. In addition, the residual ethanol content of around 20% vol/vol remained stable over one

year. Nevertheless, a loss of 33% after manufacturing with respect to the theoretical concentration (30% vol/vol) was observed. This loss was probably due to the manufacturing process since the stability study revealed a constant percentage. Loss of ethanol could have resulted in rapamycin precipitation, but microscopic analysis of the gel did not detect any undissolved rapamycin particles. In formula 2, the distribution of the α-tocopherol droplets size remained unchanged after 1 year, implying a good physical stability.

Among the different types of topical preparation based on rapamycin, only one cream containing 0.1% rapamycin solubilized in Transcutol® and mixed with a marketed cream (Excipial® hydrocream, Galderma) was studied for stability [14]. However, the stability was only achieved over 85 days while our gel is stable at 4°C for a storage of at least 1 year. These results were obtained using a highly selective and validated RP-HPLC method, allowing a good separation of isomers and degradation products including secorapamycin, an open-ring isomer with extremely low immunosuppressive activity [15].

In addition, this method has been shown to be stability indicating. The very good extraction coefficient implies the reliability of our data (92.2 ± 0.06% and 94.6 ± 0.05% for formula 1 and 2, respectively versus 64.2 ± 1.2% in Excipial® hydrocream (14)).

As shown in several studies, also based on case reports, the efficacy and tolerance of the different preparations used are more or less good depending on the rapamycin concentration, the type of raw material (API standard powders, crushed tablets or oral solutions of rapamycin), the vehicle used for the topical route and the age of the patients [11,13,16,17].

Balestri et al. analyzed 16 published reports that used a wide range of rapamycin concentration (from 0.003 to 1 %) formulated with different vehicles. They have noticed that an improvement was observed in 94% of cases, ranging from moderate to complete clearance of the lesions. The assessment of improvement varied according to the methods used, ranging from a qualitative estimate of redness and size of lesions to the use of a score (e. g. Facial Angiofibroma Severity Index: FASI). In 6% of the cases, the treatment did not improve due to a low rapamycin concentration [18].

Moreover, most authors agreed that age and clinical impairment of the patient are important factors in response to treatment. Salido et al. reported that efficacy was also better pediatric patients with growing tumors compared to adults with a median response time of 4 weeks and an average decrease in FASI of 60.2% with a rapamycin ointment of 0.4% [12]. Tu et al. also mentioned that the most striking responses were observed with younger patients presenting smaller and less developed angiofibromas [13]. Foster et al. reported that improvement in young adults was more focused on redness than established angiofibromas [19]. In fact, the skin is thinner and more permeable in children and the fibrotic and proliferative component is less, thus increasing their sensitivity to the action of sirolimus [20,21].

It should be noticing that the use of crushed tablets or rapamycin powder, dispersed into a fatty base like petrolatum or Dexeryl®, would limit the cutaneous bioavailability as the drug is not fully solubilized in the topical formulation. As a result, most authors used concentrations up to 0.1%, especially 0.2% and 1%, or increased the frequency of application (twice daily) [11,12,22,23]. During a prospective study, Malissen et al. showed a significant improvement in FASI using 1% rapamycin Dexeryl® cream, resulting in 12.5%, 40% and 50% complete clearance at 3, 6 and 9 months, with no

further benefit [23]. According to Toll et al. microspheres with less than 10 µm in size penetrate the hair follicles, which may explain some of the efficacy observed in children [24]. Moreover, bleeding was assumed to be due to incomplete crushing of the tablets. Furthermore, the oral solution was highly irritating, probably due to the high level in surfactant, making oily skin and causing poor observance and compliance [16,19].

In order to limit tolerance problems due to the presence of solid particles and to increase skin bioavailability (improved efficacy in young adults), the solubilization of rapamycin was identified as a key component of the process. Ethanol has been chosen as solubilization solvent. It is well known that ethanol is capable of improving the skin penetration of drugs (role of enhancer) by increasing the porosity of the *stratum corneum* [25]. Moreover, this latter well solubilizes rapamycin, its transcutaneous passage is facilitated. To reduce poor skin tolerance and increase patient comfort, we have added the following other excipients to our formulation: urea, glycerol and α-tocopherol. Glycerol is a hygroscopic substance used as humectant and emollient agent to promote skin hydration [26]. Moreover, topical application of glycerol improves skin elasticity and contributes to epidermal barrier repair [27]. Addition of α-tocopherol, an efficient antioxidant, was used to protect rapamycin from oxidation and also to protect the highly damaged skin of these patients, thanks to an anti-radical action [28]. Urea is traditionally used in dermatology for its moisturizing and keratolytic action [29], which can be beneficial for TSC patients with dry skin and hyperkeratosis. However, even though the low concentration used in hydro-alcoholic gels is not enough to afford a keratolytic effect, urea can still act as emollient. Based upon the good stability of the gel in the presence of urea, an increase in its concentration could be envisaged. Our formulation makes it possible to meet both the objective of treating angiofibroma, as all

studies published to date, and to improve skin tolerance by adding the above-mentioned excipients.

Finally, carbopol was added to our formulation due to an excellent tolerance and its ability to drug delivery. Moreover, carbopol gels containing penetration enhancers like ethanol have proven to be very efficacious and relatively easy to prepare. As described in Figure 3, this gel exhibited a rheofluidifiant behavior, neither affected by the presence of rapamycin nor in time.

No antimicrobial agents were added due to the presence of ethanol and the bacteriostatic and antifungal properties of rapamycin [30].

The gel-based formulation offers better application characteristics with respect to cream and ointment. In addition, as demonstrated by Tanaka et al., gel allows higher skin penetration than ointment according to *in vitro* tests performed with a three-dimensional cultured human skin model and low irritation (24). Finally, the hydrophilic character induces a better aesthetic comfort for the patient and a better observance.

Compared to formula 1, the presence of urea along with α-tocopherol in formula 2 gives it additional properties such were previously described. Moreover, in a recent study, Wataya-Kaneda et al. concluded that the optimal concentration of rapamycin hydrophilic gel was 0.2% but they observed more adverse events in the 0.2% rapamycin subgroup with skin dryness (in 62% of case) and irritation (in 50% of case). Moreover, a low blood level of rapamycin was detected in several patients especially in child subgroup (50% and 100% in adult subgroup and child subgroup respectively) leading to probable occurrence of systemic side-effects [31]. In this context, our study proposed that 0.1% appeared as the highest concentration of rapamycin allowing reaching an

excellent therapeutic index without any systemic side-effect for the patient and also for a lower cost.

CONCLUSION

The present study provides a novel gel formulation stable one year, effective against angiofibromas and safe in adulthood. The shelf-life (at least 1 year) permits the formulation in hospitals without stress and need of sophisticated materials. It is the first study to propose a formulation adapted to disease in older patient. Indeed, our excipients permit to avoid bad skin tolerance, to increase patient comfort and to increase skin bioavailability. Rapamycin was solubilized in ethanol to facilitate cutaneous penetration and various excipients were added to promote tolerance. Moreover, the hydrophilic character of the formulation induces esthetic comfort to the patient.

Finally, a clinical evaluation on a larger cohort of patients is being envisaged to evaluate the efficacy and safety of our preparation.

ACKNOWLEDGEMENT

The authors acknowledge Vincent Boudy and Benoit d'Hayer for their help during the rheological measurements and Bernard Do for its language assistance.

DISCLOSURE OF INTEREST

The authors declare that they have no conflicts of interest concerning this article.

REFERENCES

1 Ballanger, F., Quéreux, G., Stalder, J.-F., Schmitt, S., Jacquemont, S., 2005. Sclérose tubéreuse de Bourneville. EMC - Dermatol.-Cosmétologie 2, 92–102.

2 Northrup H, Krueger DA, International Tuberous Sclerosis Complex Consensus Group. Tuberous sclerosis complex diagnostic criteria update: recommendations of

the 2012 Iinternational Tuberous Sclerosis Complex Consensus Conference. *Pediatr Neurol* 2013;**49**:243–54. doi:10.1016/j.pediatrneurol.2013.08.001

3 Crall C, Valle M, Kapur K, *et al*. Effect of Angiofibromas on Quality of Life and Access to Care in Tuberous Sclerosis Patients and Their Caregivers. *Pediatr Dermatol* 2016;**33**:518–25. doi:10.1111/pde.12933

4 Schwartz, R.A., Fernández, G., Kotulska, K., Jóźwiak, S., 2007. Tuberous sclerosis complex: advances in diagnosis, genetics, and management. J. Am. Acad. Dermatol. 57, 189–202.

5 Li, S., Takeuchi, F., Wang, J., Fan, Q., Komurasaki, T., Billings, E.M., Pacheco-Rodriguez, G., Moss, J., Darling, T.N., 2008. Mesenchymal–epithelial interactions involving epiregulin in tuberous sclerosis complex hamartomas. Proc. Natl. Acad. Sci. 105, 3539–3544.

6 Darling, T.N., 2004. Hamartomas and tubers from defects in hamartin-tuberin. J. Am. Acad. Dermatol. 51, S9–11.

7 Koenig MK, Hebert AA, Roberson J, *et al*. Topical Rapamycin Therapy to Alleviate the Cutaneous Manifestations of Tuberous Sclerosis Complex. *Drugs RD* 2012;**12**:121–6. doi:10.2165/11634580-000000000-00000

8 Salido-Vallejo R, Garnacho-Saucedo G, Moreno-Giménez J c. Revisión: Opciones terapéuticas actuales para los angiofibromas faciales. *Curr Options Treat Facial Angiofibromas Engl* 2014;**105**:558–68. doi:10.1016/j.ad.2012.11.020

9 Wataya-Kaneda, M., Tanaka, M., Nakamura, A., Matsumoto, S., Katayama, I., 2011. A topical combination of rapamycin and tacrolimus for the treatment of angiofibroma due to tuberous sclerosis complex (TSC): a pilot study of nine Japanese patients with TSC of different disease severity. Br. J. Dermatol. 165, 912–916.

10 Dill, P.E., De Bernardis, G., Weber, P., Lösch, U., 2014. Topical everolimus for facial angiofibromas in the tuberous sclerosis complex. A first case report. Pediatr. Neurol. 51, 109–113.

11 DeKlotz CMC, Ogram AE, Singh S, *et al*. Dramatic improvement of facial angiofibromas in tuberous sclerosis with topical rapamycin: optimizing a treatment protocol. *Arch Dermatol* 2011;**147**:1116–7. doi:10.1001/archdermatol.2011.254

12 Salido R, Garnacho-Saucedo G, Cuevas-Asencio I, *et al*. Sustained clinical effectiveness and favorable safety profile of topical sirolimus for tuberous sclerosis - associated facial angiofibroma. *J Eur Acad Dermatol Venereol JEADV* 2012;**26**:1315–8. doi:10.1111/j.1468-3083.2011.04212.x

13 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.

14 Bouguéon G, Lagarce F, Martin L, *et al*. Formulation and characterization of a 0.1% rapamycin cream for the treatment of Tuberous Sclerosis Complex-related angiofibromas. *Int J Pharm* 2016;**509**:279–84. doi:10.1016/j.ijpharm.2016.05.064

15 Yuri V. Il'ichev, Lori Alquier, Cynthia A. Maryanoff. Degradation of rapamycin and its ring-opened isomer: Role of base catalysis. *ARKIVOC* 2007;**2007**:110–31.

16 Mutizwa MM, Berk DR, Anadkat MJ. Treatment of facial angiofibromas with topical application of oral rapamycin solution (1mgmL(-1)) in two patients with tuberous sclerosis. *Br J Dermatol* 2011;**165**:922–3. doi:10.1111/j.1365-2133.2011.10476.x

17 Wheless JW, Almoazen H. A novel topical rapamycin cream for the treatment of facial angiofibromas in tuberous sclerosis complex. *J Child Neurol* 2013;**28**:933–6. doi:10.1177/0883073813488664

18 Balestri R, Neri I, Patrizi A, *et al*. 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* 2015;**29**. doi:10.1111/jdv.12665

19 Foster RS, Bint LJ, Halbert AR. Topical 0.1% rapamycin for angiofibromas in paediatric patients with tuberous sclerosis: a pilot study of four patients. *Australas J Dermatol* 2012;**53**:52–6. doi:10.1111/j.1440-0960.2011.00837.x

20 Vasani RJ. Facial Angiofibromas of Tuberous Sclerosis Treated with Topical Sirolimus in an Indian Patient. *Indian J Dermatol* 2015;**60**:165–9. doi:10.4103/0019-5154.152516

21 B R-N, M A-C, P M-P, *et al*. Topical rapamycin in facial angiofibromas: patients satisfaction versus objective response, and a literature review. *Clin Dermatol*;**Vol. 2**:93–8. doi:10.11138/cderm/2014.2.2.093

22 Haemel AK, O'Brian AL, Teng JM. Topical Rapamycin: A Novel Approach to Facial Angiofibromas in Tuberous Sclerosis. *Arch Dermatol* 2010;**146**:715–8. doi:10.1001/archdermatol.2010.125

23 Malissen N, Vergely L, Simon M, *et al*. Long-term treatment of cutaneous manifestations of tuberous sclerosis complex with topical 1% sirolimus cream: A prospective study of 25 patients. *J Am Acad Dermatol* doi:10.1016/j.jaad.2017.04.005

24 Toll R, Jacobi U, Richter H, *et al*. Penetration Profile of Microspheres in Follicular Targeting of Terminal Hair Follicles. *J Invest Dermatol* 2004;**123**:168–76. doi:10.1111/j.0022-202X.2004.22717.x

25 Williams AC, Barry BW. Penetration enhancers. *Adv Drug Deliv Rev* 2004;**56**:603–18. doi:10.1016/j.addr.2003.10.025

26 Hara M, Verkman AS. Glycerol replacement corrects defective skin hydration, elasticity, and barrier function in aquaporin-3-deficient mice. *Proc Natl Acad Sci U S A* 2003;**100**:7360–5. doi:10.1073/pnas.1230416100

27 Fluhr JW, Darlenski R, Surber C. Glycerol and the skin: holistic approach to its origin and functions. *Br J Dermatol* 2008;**159**:23–34. doi:10.1111/j.1365-2133.2008.08643.x

28 Nachbar F, Korting HC. The role of vitamin E in normal and damaged skin. *J Mol Med Berl Ger* 1995;**73**:7–17.

29 Friedman AJ, von Grote EC, Meckfessel MH. Urea: A Clinically Oriented Overview from Bench to Bedside. *J Drugs Dermatol JDD* 2016;**15**:633–9.

30 Vézina C, Kudelski A, Sehgal SN. Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle. *J Antibiot (Tokyo)* 1975;**28**:721–6.

31 Wataya-Kaneda M, Nakamura A, Tanaka M, *et al.* Efficacy and Safety of Topical Sirolimus Therapy for Facial Angiofibromas in the Tuberous Sclerosis Complex : A Randomized Clinical Trial. *JAMA Dermatol* 2017;**153**:39–48. doi:10.1001/jamadermatol.2016.3545

32 Tanaka, M., Wataya-Kaneda, M., Nakamura, A., Matsumoto, S., Katayama, I., 2013. First left-right comparative study of topical rapamycin vs vehicle for facial angiofibromas in patients with tuberous sclerosis complex. Br. J. Dermatol. 169, 1314–1318.