Topical rapamycin combined with pulsed dye laser in the treatment of capillary vascular malformations in Sturge-Weber syndrome: Phase II, randomized, double-blind, intraindividual placebo-controlled clinical trial

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Background: Sturge-Weber syndrome (SWS) is characterized by port-wine stains (PWS) affecting the face, eyes, and central nervous system. Pulsed dye laser (PDL) is the standard treatment for PWS. Unfortunately, recurrence is frequent because of reformation and reperfusion of blood vessels.

Objective: We sought to assess the clinical efficacy of topical rapamycin combined with PDL in PWS of patients with SWS.

Methods: We conducted a phase II, randomized, double-blind, intraindividual placebo-controlled, clinical trial. We recruited 23 patients with SWS and facial PWS (12 women; median age 33 years, age range 17-65 years) from the University Clinic of Navarra, Spain. Four interventions were evaluated: placebo, PDL + placebo, rapamycin, and PDL + rapamycin. Clinical and histologic responses were evaluated using a chromatographic computerized system, spectrometry, and histologic analyses at 6, 12, and 18 weeks after the intervention.

Results: PDL + rapamycin yielded the lowest digital photographic image score and the lowest percentage of vessels in histologic analysis, and showed a statistically significant improvement compared with the other interventions. The treatment was generally well tolerated.

Limitations: PDL was only applied to the lateral parts of the PWS area.

Conclusion: Topical rapamycin associated with PDL seems to be an effective treatment for PWS in patients with SWS. (J Am Acad Dermatol 2015;72:151-8.)

Key words: capillary vascular malformation; pulsed dye laser; rapamycin; Sturge-Weber syndrome.

Sturge-Weber syndrome (SWS) is a congenital multisystem disorder characterized by ipsilateral capillary-venous malformation of the face (port-wine stain [PWS] birthmark), choroidal and episcleral vascular malformation, and leptomeningeal malformation.[1,2]

Abbreviations used:

HIF:	hypoxia-inducible factor
OV:	office visit
PDL:	pulsed dye laser
PWS:	port-wine stain
SWS:	Sturge-Weber syndrome

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Facial PWS in SWS are well-demarcated red or violaceous macules typically involving the skin area roughly corresponding to that innervated by the ophthalmic branches of the trigeminal nerve, and, occasionally, the second and third branches.[3] Histopathologic examination of PWS in SWS shows dilatation of the capillaries and postcapillary venules of the superficial vascular plexus. Progressive blood vessel ectasia may be related to a deficiency in sympathetic innervation of the vessel and the failure to regulate vasoconstriction.[4,5] These lesions may lead to psychosocial morbidity.[6,7]

Pulsed dye laser (PDL) remains the standard treatment for PWS,[8,9] although complete resolution is rare[10] despite multiple sessions.[11] The regeneration and revascularization of photocoagulated blood vessels after treatment may contribute to these unsatisfactory results.[12]

CAPSULE SUMMARY

- Pulsed dye laser remains the standard treatment for port-wine stain.
- Topical rapamycin improves the results of laser treatment, probably by inhibiting laser-induced neoangiogenesis, and reduces the total number of required sessions.
- Topical rapamycin associated with pulsed dye laser seems to be an effective treatment for port-wine stain in patients with Sturge-Weber syndrome.

Rapamycin is an immunosuppressive agent with the capacity to inhibit mammalian target of rapamycin (mTOR)-mediated functions (protein synthesis, cell proliferation, and tumor angiogenesis).[13,14] A study using an animal model showed that PDL induced an increase in the messenger RNA and protein levels of hypoxia-inducible factor (HIF)-1α, vascular endothelial growth factor, and phosphorylated ribosomal protein S6 (pS6), a protein activated at the end of the mTOR pathway, suggesting that angiogenesis pathways play an active role in the skin blood vessel regeneration and revascularization.[15] Topical application of rapamycin suppressed the PDL-induced increase in messenger RNA, HIF-1α, vascular endothelial growth factor, and pS6 levels.[16]

We evaluated the efficacy and safety of topical rapamycin alone and combined with PDL in the treatment of PWS of patients with SWS.

METHODS

Study design

This is a phase II, randomized, double-blind, intraindividually placebo-controlled clinical trial to assess the efficacy and safety of topical rapamycin associated with PDL in patients with SWS.

The study protocol was approved by the institutional review board of the University of Navarra and by the Spanish Agency of Drugs and Sanitary Products, and it was registered in the European Union Clinical Trials Register (2010-024078-20) and Clinical Trials.gov (NCT02080624).

Participants and study setting

Patients with SWS were recruited in the University Clinic of Navarra (Navarra, Spain), Hospital Ramón y Cajal (Madrid, Spain), Instituto Valenciano de Oncología (Valencia, Spain), and Hospital de Sant Pau i de la Santa Creu Hospital (Barcelona, Spain). Verbal and written informed consent was obtained from all patients or from the legally authorized representative.

Interventions

Four interventions were evaluated: placebo, PDL + placebo, rapamycin, and PDL + rapamycin (Fig 1). Patients were evaluated on 4 occasions: office visit (OV) 0 (baseline), OV1 (6 weeks after OV0), OV2 (12 weeks after OV0), and OV3 (18 weeks after OV0).

Laser treatment

All patients received laser treatment using PDL (Cynergy, Cynosure Inc, Westford, MA) with the following parameters: fluence, 9 J/cm^2; pulse width, 2 milliseconds; wavelength, 595 nm; single pass; spot size, 7 mm; with Smartcool cooling system (Cynosure Inc). Following an individualized stencil, laser treatment was applied to the lateral parts of the PWS at OV0 and OV1.

Topical treatment

After the treatment with PDL at OV0, all patients began daily treatment with 0.5 g of 1% rapamycin cream and with 0.5 g of control cream (placebo) on the superior and inferior half of the PWS depending on the randomized assignment, for 12 weeks.

The topical rapamycin formulation contained 1% rapamycin powder dissolved in 3.8% benzyl alcohol and thoroughly mixed in a water-in-oil emulsion. This formulation was fabricated following good manufacturing practices (European United Federal Law: 91/356/CEE [Community European Economy]) in an authorized pharmaceutical laboratory (Laboratorium Sanitatis, Vitoria, Spain). The vehicle used in the topical rapamycin formulation was exactly the same cream used in the manufacture of the control cream.

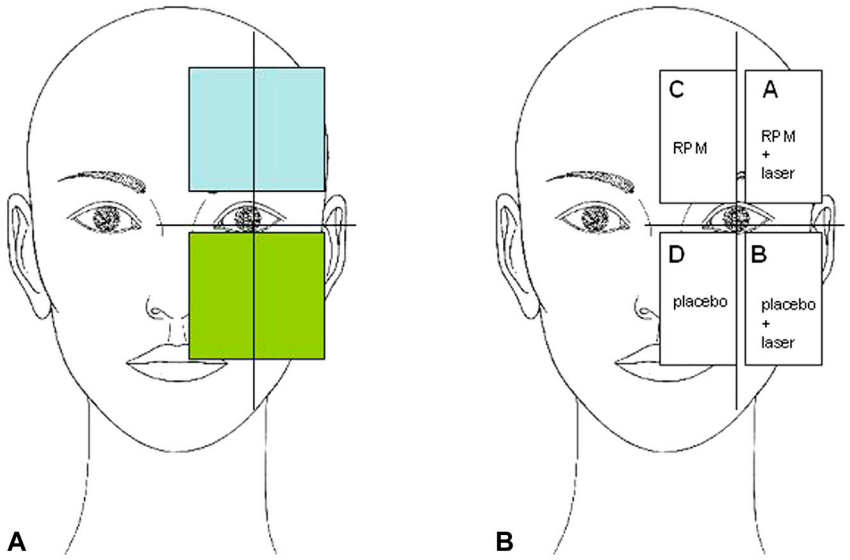

Fig 1. Each patient's facial port-wine stain (PWS) area was divided into 4 parts (superolateral [*A*], inferolateral [*B*], superomedial [*C*], inferomedial [*D*]). Pulsed dye laser was applied to the lateral parts (superolateral and inferolateral) and topical rapamycin (*RPM*) vs placebo treatment was randomly assigned to the superior or inferior parts. There were 2 types of syringes with cream: the blue and the green ones; patients were advised to always put the blue ones in the superior part and the green ones in the inferior part following the stencil (**A**). In the example case, the blue syringes had RPM (**B**). This design let us treat the PWS with 4 different treatments: RPM, RPM + laser, placebo, and laser + placebo.

Outcome assessment

To determine the efficacy of these treatments, we designed a morphologic and chromatographic computerized system used in combination with spectrometry, histologic studies, and immunohistochemistry. In addition, 4 dermatologists subjectively evaluated the improvement of the stain using a 5-point scale, based on changes in color and boundary of the stain at OV1, OV2, and OV3. The reliability between observers was evaluated by estimating the global kappa statistic of agreement and its 95% confidence interval based on the jackknife technique. The safety and pharmacokinetics of topical 1% rapamycin were assessed by analyzing blood samples, measuring rapamycin blood levels, and using tolerability tests.

Photographic images analysis software

A program was designed as a plugin for FIJI (a distribution of ImageJ, Rasband WS, US National Institutes of Health, Bethesda, MD)[17,18] (Appendix, available at http://www.jaad.org).

Histology

Punch biopsy specimens were taken from each of the 4 different treated parts at OV2. Immunohistochemical staining for Ki67, CD31, D2-40, nestin, and phosphoS6 was performed. To count the number of the vessels in each area, the photographs of CD31 staining samples images were captured at 20X (Plan-Neofluar objective with 0.50NA) magnification with an AxioImager.M1 microscope (Zeiss, Oberkochen, Germany) connected to a Spot Insight 2.0 Color camera (Spot imaging solutions, Burroughs, Sterling Heights, MI). The software detects CD31 by performing color segmentation in the hue-saturation-brightness color model and a set of filters improves the detection quality, eliminating false positives by size.

The rest of the immunochemical analysis was evaluated by analyzing the specimen's degree of staining depending on the proportion of cells stained and their intensity into 1 of 4 categories: 1 (0%-25%), 2 (25%-50%), 3 (50%-75%), and 4 (>75%).

Blood tests

The 1% rapamycin blood bioavailability was analyzed during its administration at OV1 and OV2 (chemiluminescent microparticle immunoassay technique, Architect Abbott immunochemistry system, Abbott Laboratories, Abbott Park, IL). Complete blood cell counts were performed and triglyceride and cholesterol levels were also measured at OV0, OV1, OV2, and OV3.

Tolerability test

Local tolerability (irritancy) of the treatment was assessed at each visit using a Frosch and Kligman[19] visual scale.

Table I. Efficacy of interventions evaluated by digital photographic image and histology analyses at different patients visits

	Treatment area								
	PDL + placebo		Placebo		PDL + 1% RPM		1% RPM		
	Median	p25; p75	Median	p25; p75	Median	p25; p75	Median	p25; p75	*P* value[†]
Digital photographic image analysis[*]									
OV0	16.9	12.9; 19.9	16.8	15.0; 21.0	16.1	10.7; 18.5	16.5	13.2; 21.0	.508
OV1	13.5	12.0; 18.0	15.8	14.3; 22.0	11.8	8.7; 13.7	15.1	12.1; 20.4	.001
OV2	12.7	10.8; 16.7	15.9	12.9; 21.4	8.9	5.7; 13.7	15.0	11.1; 20.8	<.001
OV3	12.9	11.1; 17.5	16.6	13.5; 21.6	9.3	5.6; 11.5	15.0	11.1; 21.2	<.001
Vascular area[‡]									
OV2	8.0	6.6; 10.9	10.1	8.2; 13.1	6.4	3.8; 8.3	10.5	8.0; 13.1	<.001

OV0, Baseline visit (commencement of intervention); *OV1*, 6 weeks after OV0; *OV2*, 12 weeks after OV0; *OV3*, 18 weeks after OV0; *p25*, 25th percentile; *p75*, 75th percentile; *PDL*, pulse dye laser; *RPM*, rapamycin.

*Expressed as digital photographic image score (from 0 [lowest] to 100 [greatest] intensity of the port-wine stain erythema).

[†]Friedman test.

[‡]Expressed as percentage of vessels.

Sample size

To detect the difference in the intensity of the PWS erythema of 0.6 U according to the digital photographic image score, using the smallest expected difference between 2 intervention areas, assuming an overall correlation of 0.7, with a 2-sided 5% significance level and a power of 80%, and given an anticipated dropout rate of 10%, 23 patients were necessary.

Randomization

The process was designed and executed by an external centralized randomization service formed by staff with no clinical involvement in the trial.

Statistical analyses

All analyses were performed using SPSS Statistics 20 (IBM Corp, Armonk, NY), Stata 12 (StataCorp LP, College Station, TX), and EPIDAT 3.1 (Dirección Xeral de Saúde Pública, Xunta de Galicia, A Coruña, Spain). The Friedman test was used to compare efficacy and security parameters according to interventions at OV0, OV1, OV2, and OV3. *P* values less than or equal to .05 were considered to indicate statistical significance, using 2-sided tests. All comparisons were conducted on an intent-to-treat basis. Post hoc analysis with Wilcoxon matched pairs signed rank tests was conducted applying Bonferroni correction for multiple comparisons that established a significance level set at *P* value less than or equal to .017.

RESULTS

Baseline characteristics of the participants

In all, 23 patients with SWS and facial PWS were enrolled in the study (12 women; median age 33 years, age range 17-68 years; all patients were Caucasian, with skin types II and III). The facial PWS was localized on the right side (n = 7 patients), on the left side (n = 9), and on both sides (n = 7) of the face. The distribution for the unilateral PWS was: following the first and second trigeminal branches (n = 15) and following the 3 branches (n = 1), and for the bilateral PWS was: 3 branches on both sides (n = 1), 3 branches on 1 side and 2 on the other side (n = 3), and 3 branches on 1 side and 1 on the other side (n = 3). Nine of 23 had extrafacial PWS. Most patients had undergone previous treatments with PDL (n = 21), with an average of 25 sessions; 12 patients had tried another type of laser (argon, neodymium:yttrium-aluminium-garnet, carbon-dioxide); and 4 patients had received other treatments (cryotherapy, surgery, radiotherapy, peelings). Thirteen patients presented with scars and 15 patients had hypertrophy of the PWS.

Efficacy assessment

Baseline analysis of digital photographic images showed no differences among the 4 intervention areas of the PWS (Table I) (visit OV0; $P = .508$). Overall, we found statistically significant differences in the digital photographic image scores among the 4 interventions areas in measurements carried out at OV1 ($P = .001$), OV2 ($P < .001$), and OV3 ($P < .001$). At all evaluation visits, the combination of PDL and rapamycin was found to be the intervention achieving the lowest digital photographic image score (Table I). Post hoc pairwise analyses between the PDL + rapamycin treatment and each of the remaining therapeutic approaches showed statistically significant differences in digital photographic

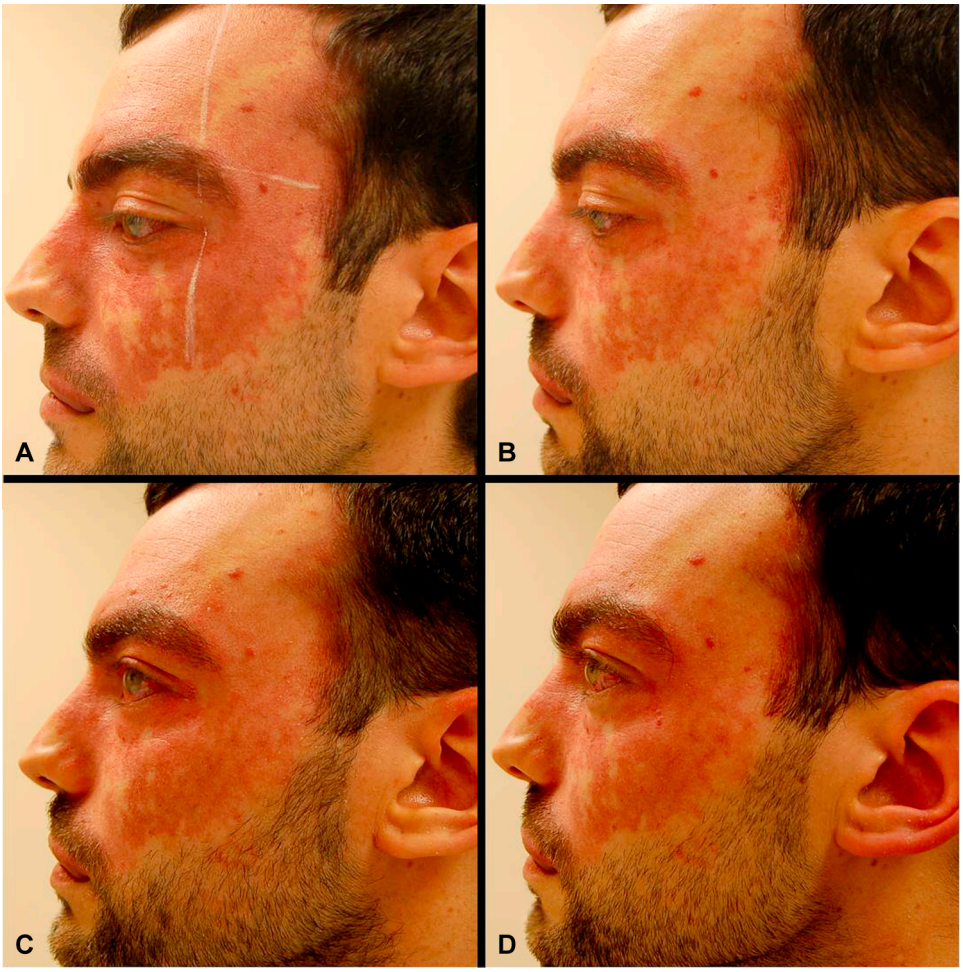

Fig 2. Photographs taken before treatment (**A**), after 6 weeks (OV1) (**B**), and after 12 weeks (OV2) (**C**); posterior control at 18 weeks (OV3) (**D**). The lateral part of port-wine stain was treated with laser, and in this patient, according to the randomization, the rapamycin treatment was applied in the superior half. Note an important clinical subjective improvement in OV1 (**B**) and in OV2 (**C**) in the part treated with rapamycin + laser, and less improvement in the part treated with laser alone. This improvement in the rapamycin + laser part seems to persist in time from OV2 (**C**) until OV3 (**D**). The other 2 parts did not demonstrate improvement.

image score results at OV1 (PDL + rapamycin vs placebo, P = .001; PDL + rapamycin vs PDL + placebo, P = .007; PDL + rapamycin vs rapamycin, P = .002), at OV2 (PDL + rapamycin vs placebo, P value < .001; PDL + rapamycin vs PDL + placebo, P = .001; PDL + rapamycin vs rapamycin, P = .001), and at OV3 (PDL + rapamycin vs placebo, P < .001; PDL + rapamycin vs PDL + placebo, P = .002; PDL + rapamycin vs rapamycin, P = .002) (Fig 2). The global kappa statistic of agreement among the 4 dermatologists who assessed subjectively the improvement of the stain was 0.98 (95% confidence interval 0.964-0.996).

Histologic analyses carried out at OV2 revealed statistically significant differences in the percentage of vessels between interventional areas (Table I) (P < .001). PDL + rapamycin showed the lowest percentage of vessels, compared with the rest of the interventions, and presented statistically significant differences with each of these in post-hoc pairwise analyses (PDL + rapamycin vs placebo, P = .001; PDL + rapamycin vs PDL + placebo, P = .002; PDL + rapamycin vs rapamycin, P = .001) (Fig 3).

The changes occurring in digital photographic image scores from the baseline assessment to each subsequent evaluation are displayed in Table II. The greatest reduction was observed in the PDL + rapamycin interventional area in all evaluations. Post hoc pairwise comparisons between the PDL + rapamycin treatment area and the other 3 treatment zones produced the following results: from OV0 to OV1 (PDL + rapamycin vs placebo,

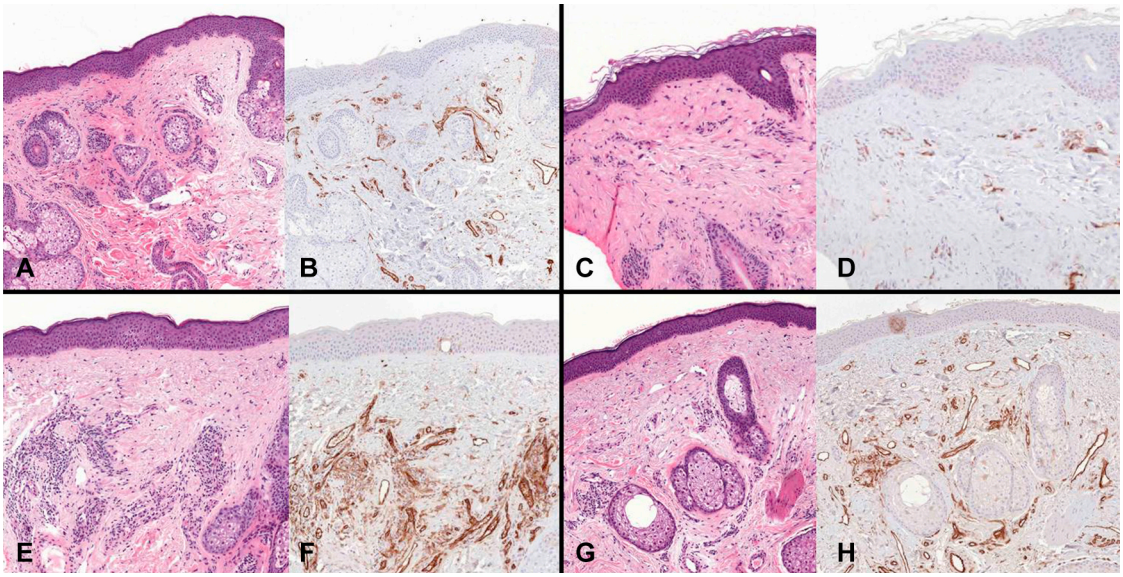

Fig 3. Hematoxylin-eosin and CD31 stains. We used CD31 stain to count the number of vessels that exist in each area and calculated the vascular area (VA; VA = space per vessel/dermal area). Zones treated with: rapamycin: VA 7.97% (**A** and **B**); laser + rapamycin: VA 1.17% (**C** and **D**); rapamycin: VA 8.70% (**E** and **F**); and rapamycin: VA 5.83% (**G** and **H**). Note that laser + rapamycin sample has fewer vessels in both stains.

Table II. Changes in digital photographic image scores according to interventional area

Digital photographic image score change	Treatment area								
	PDL + placebo		Placebo		PDL + 1% RPM		1% RPM		
	Median	p25; p75	Median	p25; p75	Median	p25; p75	Median	p25; p75	*P* value*
OV1-OV0	−1.1	−2.3; 0.4	0.1	−1.7; 1.0	−4.1	−5.0; −2.0	−0.1	−1.6; 0.7	.003
OV2-OV0	−1.9	−4.1; 0.1	−0.7	−1.5; 0.5	−5.7	−7.9; −3.8	−0.4	−1.3; 0.5	<.001
OV3-OV0	−1.5	−3.9; 0.4	−0.2	−1.3; 1.0	−5.5	−7.6; −4.8	−0.1	−0.9; 0.6	.002

OV0, Baseline visit (commencement of intervention); *OV1*, 6 weeks after OV0; *OV2*, 12 weeks after OV0; *OV3*, 18 weeks after OV0; *p25*, 25th percentile; *p75*, 75th percentile; *PDL*, pulse dye laser; *RPM*, rapamycin.
*Friedman test.

P = .001; PDL + rapamycin vs PDL + placebo, P = .001; PDL + rapamycin vs rapamycin, P = .004); from OV0 to OV2 (PDL + rapamycin vs placebo, P value < .001; PDL + rapamycin vs PDL + placebo, P < .001; PDL + rapamycin vs rapamycin, P = .003); and from OV0 to OV3 (PDL + rapamycin vs placebo, P < .001; PDL + rapamycin vs PDL + placebo, P < .001; PDL + rapamycin vs rapamycin, P = .007).

Results from the analysis of the proportion and the intensity of cells stained with nestin, ki67, and phosphorylated S6 did not reveal any significant difference among the 4 interventional areas.

All patients described a subjective improvement of their lesions that was rated as very slight improvement (13% of the patients at 6 weeks, and 13% at 12 weeks), slight (60.9% at 6 weeks, and 21.7% at 12 weeks), considerable (21.7% at 6 weeks, and 52.2% at 12 weeks), or very significant (4.3% at 6 weeks, and 13% at 12 weeks).

Safety and tolerability evaluation

Neither blood analyses at baseline or blood examinations carried out at OV1 showed any abnormalities.

The median blood concentration of rapamycin was 0.69 ng/mL (25th percentile: 0.45 ng/mL; 75th percentile: 0.95 ng/mL) at OV1 and 1.07 ng/mL at OV2 (25th percentile: 0.91 ng/mL; 75th percentile: 1.31 ng/mL). One patient showed blood rapamycin concentration values that were more than 1.5 times the value of the interquartile range (75th percentile-25th percentile) above the third quartile (75th percentile) at OV1 (3.39 ng/mL) and at OV2 (2.46 ng/mL).

The intervention was generally well tolerated by all subjects. The detected side effects were: mild facial acne (n = 8 patients), small canker sores (n = 3), herpes labialis (n = 1), transient numbness of the upper lip (n = 4), and slight and temporary stinging in the treated zone after the application of the cream (n = 14). According to the Frosch and Kligman[19] visual scale, 5 patients experienced fine scaling at OV1, but neither erythema nor fissuring were observed. Nevertheless, 1 patient presented with moderate contact dermatitis at 7 weeks.

DISCUSSION

This clinical trial demonstrates that treatment with topical rapamycin in combination with PDL in the PWS of patients with SWS is more effective than laser treatment on its own and the treatment-associated adverse events were minor.

PDL laser is currently the treatment of choice for capillary malformations. However, the extent to which these lesions improve varies widely and complete disappearance is not frequent; 1 study showed only 2% of patients experienced complete disappearance after a mean number of 17 treatments.[20]

According to published reports these results are even worse in PWS of patients with SWS.[21] It has been postulated that the treatment failure of PWS lesions is in part a result of regeneration and revascularization of photocoagulated vessels.[14] Blood supply to the skin is markedly reduced after laser photothermolysis-induced vessel coagulation. Resultant local hypoxia stimulates the formation of HIF-1α, which in turn induces the expression of multiple proangiogenic factors such as vascular endothelial growth factor, the presence of which stimulates the mTOR pathway.[22] The mTOR pathway is a well-known primary activator of HIF-1α. Consequently, mTOR can phosphorylate the S6, which then mediates efficient cap-dependent translation initiation and finally results in neoangiogenesis.[23-25]

Rapamycin exerts a strong antiangiogenic effect by inhibiting the intracellular pathways that are activated after the damage caused by the laser and that induce neoangiogenesis.[13,14] Indeed, rapamycin has recently been described as a good treatment for hypervascular anomalies including angiomyolipomas, Kaposi sarcoma, psoriasis, and angiofibromas.[26-29]

Nelson et al[30] reported an increased effectiveness of PDL in patients with PWS treated with oral rapamycin that persisted at 13 months. Although in our trial no subsequent physical examination was included, 13 patients were monitored by telephone 3 to 6 months after the end of the study and no subjective worsening was reported.

The low effectiveness of the administration of rapamycin alone, without any associated laser treatment, may be explained by the absence of active angiogenesis in the natural progression of PWS in patients with SWS.

Although we found evidence of the systemic absorption of the drug, we observed no analytical or clinical abnormalities associated with its use. This observation is inconsistent with other reports that have found no systemic absorption of the drug after topical application for other dermatologic disorders.[28,29] This increased absorption may be a result of the higher concentrations of the drug used or to the fact that capillary vascular malformations are highly vascularized lesions. Curiously, the 2 outliers who presented the highest levels consumed considerable daily amounts of soy, a CYP3A4 inhibitor and a liver enzyme involved in the metabolism of rapamycin. We did not observe any association between clinical efficacy and rapamycin blood levels.

Some limitations to our study deserve mention. First, PDL was only applied to the lateral parts of the PWS area (not randomly applied to either medial or lateral parts). Second, the observed therapeutic efficacy cannot be guaranteed to last beyond the follow-up time (18 weeks). Third, all the patients were adults. It is well known that PWS become thicker with age, that the depth reached by the PDL laser is less than 1.5 mm, and that with PDL the greatest clinical improvement is seen in the first 5 treatment sessions.[31] Consequently, laser treatment is more effective in thinner lesions, usually at younger ages. Furthermore, patients with SWS present greater hypertrophy than patients with nonsyndromic PWS and thus PDL treatment in such patients will be much less effective. The patients in our trial had previously received an average of 15 PDL sessions. It is believed that the decreasing effectiveness is a result of destruction of the smaller, more superficial capillaries with the initial PDL treatments, leaving the deeper, larger capillaries, which are more difficult to treat.[32,33]

Treatment with PDL laser in combination with topical rapamycin may be even more effective in pediatric patients. Although its systemic absorption does not reach levels comparable with that observed after oral administration, it would be advisable to perform clinical trials with pediatric patients to assess its safety in this group of patients.

In conclusion, our study suggests that topical rapamycin has no remarkable adverse effects, improves the results of laser treatment, and reduces

the total number of required sessions. In more refractory patients, it would be useful to assess the effect of combining rapamycin with neodymium:yttrium-aluminium-garnet laser or increasing the pulse duration with PDL, treatments that are more appropriate for ectatic vessels of greater caliber and those deeper in the skin.[34,35] Further research is warranted to investigate if this beneficial effect may be expanded to patients with PWS that are not associated with SWS.

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APPENDIX

Photographic images analysis software

For the measurements, a program was designed as a plugin for FIJI (a distribution of ImageJ).[17] Standardized digital photographs (Canon D500, Canon, Tokyo, Japan) of each patient were taken at every office visit (OV) (OV0 [baseline], OV1 [6 weeks after OV0], OV2 [12 weeks after OV0], and OV3 [18 weeks after OV0]) with the patient in the same position and under the same lighting conditions. The software starts by creating a stack with the images and aligning the faces. It then measures the differences in color between 2 healthy zones (a reference and a control) and 4 port-wine stain (PWS) zones in the first photograph (a zone from each of the 4 different treated areas) and follows the progression of these differences in time by comparison with subsequent photographs. If the PWS improves during the treatment, the differences in color between healthy zones and PWS should decrease among times OV0, OV1, and OV2, and should remain constant between times OV2 and OV3 (when no treatment was given). For this analysis, the software transforms every image to the CieLab color field (a color-opponent space with dimension L for lightness and a and b for the color-opponent dimensions, based on nonlinearly compressed CIE XYZ color space coordinates) and measures the L* (ranging from 0 [black] to 100 [diffuse white]), a* (−60 [red/magenta] to +60 [green]), and b* (−60 [yellow] to +60 [blue]) mean values. Uniform changes of components in the relative perceptual differences between any 2 colors in L*a*b* can be approximated by treating each color as a point in a 3-dimensional space and taking the Euclidean distance between them $-\Delta E = \mathrm{sqrt}[(\Delta L^*)2 + (\Delta a^*)2 + (\Delta b^*)2]$.[18] The program calculates the Euclidean distances of every area and the distances of the combinations of a* and b* ($\Delta E^* = \mathrm{sqrt}[(\Delta a^*)2 + (\Delta b^*)2]$). This last measurement seems to be quite accurate when comparing colors in digital photographs and eliminates the influence of lighting.