Efficacy and safety of propranolol gel for infantile hemangioma: A randomized, double-blind study

Naoaki Rikihisa\textsuperscript{a,*}, Hirokazu Takatsuka\textsuperscript{b}, Takaaki Suzuki\textsuperscript{b,c}, Yuki Shiko\textsuperscript{d}, Yohei Kawasaki\textsuperscript{d}, Michiko Hanawa\textsuperscript{d}, Itsuko Ishii\textsuperscript{b,c} and Nobuyuki Mitsukawa\textsuperscript{a,e}

\textsuperscript{a}Department of Plastic, Reconstructive and Aesthetic Surgery, Chiba University Hospital, Chiba, Japan;
\textsuperscript{b}Division of Pharmacy, Chiba University Hospital, Chiba, Japan;
\textsuperscript{c}Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan;
\textsuperscript{d}Biostatistics Section, Clinical Research Center, Chiba University Hospital, Chiba, Japan; and
\textsuperscript{e}Department of Plastic, Reconstructive, and Aesthetic Surgery, Chiba University Graduate School of Medicine, Chiba, Japan

Corresponding Author:
Naoaki Rikihisa M.D., Ph.D.
Oyumino Central Hospital
6-49-9 Oyumino-minami, Midori-ku, Chiba, Japan 266-0033
E-mail address: rikihisa@faculty.chiba-u.jp
Summary

We aimed to evaluate the efficacy and safety of propranolol gel at various concentrations with infantile hemangiomas after proliferative phases. We designed a single-center, randomized, double-blind, dose-dependent trial with placebo control and randomized patients to receive propranolol gel at 0%, 1%, or 5%, twice daily for 24 weeks. The primary efficacy endpoint was the percentage change in redness of the tumors. Safety endpoints were skin characteristics changes and systemic symptoms. We made two comparisons to evaluate the superiority of 1% and 5% propranolol gels against placebo for primary endpoint analysis and used the t-test to compare parents’ satisfaction with these treatments. Initially, 19 patients were enrolled, but 8 were excluded from the analysis. We were underpowered to answer the question of efficacy. In the per-protocol set, we found similar results for the redness percentage change among the patients on placebo, 1% and 5% gel. However, the difference in redness before and after treatment suggested a slight decreasing trend of lesion’s redness as the propranolol concentration increased. The difference in parents’ satisfaction between the placebo and 5% propranolol gel groups was significant ($p = 0.08$). We observed no serious adverse events. We did not find an obvious dose-dependent effect for the propranolol gel treatment against infantile hemangiomas after the proliferative phase. However, external applications twice daily were less burdensome for parents and led to good compliances. It had a favorable safety profile in Japanese pediatric patients with infantile hemangiomas.

Keywords: Infantile hemangioma, topical treatment, propranolol gel
Introduction

Infantile hemangiomas (IH) are neoplastic proliferative lesions of the vascular endothelium, which are also called strawberry marks. The lesions grow until 6–8 months after birth during the proliferative phase, but then shrink during the involuting phase.\(^1\) Oral propranolol treatment reduces infantile hemangiomas (IH) in >80% of patients.\(^3\) Long-term oral propranolol treatment is used to avoid serious complications (i.e., sudden death) resulting from hypertrophic cardiomyopathy.\(^8\) Although the risk of this is small, being vigilant against hypoglycemia in children receiving chronic β-blocker therapy is essential, particularly when their calorie intake is reduced for any specific reason.\(^9\)

Neonates treated with oral propranolol show plasma propranolol concentrations above 20 ng/ml in cases with serious adverse effects. Therefore, this is currently considered the maximum safe dosage.\(^10\) When retinopathy of prematurity is treated with external propranolol, plasma levels of propranolol have been found to not only be in the order of magnitude lower than that after oral treatment but also below 20 ng/ml.\(^10\) External treatments for IH may have additional benefits such as allowing a reduction in β-blocker dosage and increasing parental treatment compliance.\(^3\) The American Academy of Pediatrics Clinical Practice Guideline for IH had suggested clinicians to prescribe topical timolol maleate for thin superficial IH previously.\(^3\) The use of topical propranolol has been described in few studies.\(^20\)\(^-\)\(^23\)

We evaluated whether changing the concentration of propranolol in the gel results in different effects according to the following outcomes: Bringing the color tone of the lesion closer to that of healthy skin, reducing the β-blocker dose and the complication risk and improving parents’ compliance are goals that need to be achieved to establish a new topical treatment for IH after the proliferative phase.
Subjects and Methods

Study design
We conducted this randomized, double-blind, placebo-controlled, parallel group study (approval no. CRB3180015) with approval from our university hospital’s clinical research center.

Study population

Diagnostic criteria
All IH diagnoses were based on the criteria of the American Academy of Pediatrics Clinical Practice Guideline for the Management of IH.(5)

Inclusion and exclusion criteria
We enrolled Japanese patients aged 2–15 years with a superficial type of IH to exclude patients in proliferative phase. By interviewing the parents, we excluded patients with asthma, cardiac dysfunction, severe liver and kidney dysfunctions and thyroid poisoning. We also excluded patients using adrenaline, β-blockers, and steroids. In addition, we excluded children with dry skin, papules, scaling, ulcers, and bleeding in the affected area; patients being treated with propranolol oral treatment and patients deemed unsuitable by attending physicians.

Case registration, randomization, and blinding
The investigator obtained written consents to screen patients. Patients were registered before the start of the study treatment protocol. We entered information required for case registration into the data system at our University Hospital Data Center, and we randomly assigned patients to receive propranolol gel at 0 (placebo), 1% or 5% using a computer application. The stratified randomization factors for allocation included the lesion area (less than 10 cm², over 10 cm²). The block randomization was also used to keep sample size equivalent between arms. The randomization results were communicated only to the pharmacist who dispensed the study medications.

Interventions
Propranolol and placebo gels were applied externally, twice daily for 24 weeks. Specifically, the gel was gently spread over the whole tumor over 5 s with a small-head-size gel per 200 cm². Propranolol and placebo gels were produced and packaged at the Division of Pharmacy, our University Hospital. Studies using 1% propranolol ointment have been frequent(20-23) and a report on the safety and efficacy of 3% propranolol (highest concentration at the planning this study) also exists.(20-23) Thus, we dispensed 0.2 g (1% w/v) and 1 g (5% w/v) of propranolol hydrochloride (Tokyo Chemical Industry, Japan) into a beaker suspended over 70°C phosphate-buffered saline (pH 7.4). While stirring, we
added 0.2 g/20 mL of hydrophobically modified hydroxy-propyl-methyl-cellulose (HM-HPMC, Sangelose® 60L, Daido Chemical) to obtain a uniform suspension. The suspension was cooled and then stirred until it became transparent. We made the placebo gel by omitting propranolol from the recipe.

**Outcome measures assessments**

We assessed and recorded all outcome measures at baseline (0) and at every 4 weeks until 24 weeks of treatment. In addition, we conducted follow-up assessments after the end of treatment. We recorded the following information at the time of the screening test: consent acquisition date, identification, patient’s background, including gender, year of birth, age, body height and weight, medical history in data center using a double-blinded approach.

At 0, 4, 8, 12, 16, 20 and 24 weeks of treatment and follow-up assessments, we evaluated: lesion area, status of external applications during the period, vital signs, weight and height, use of concomitant medications, presence of adverse events, such as contact dermatitis, itching or atrophy.

We obtained blood samples for a blood count and biochemistry lab tests at the time of the screening test and 24 weeks after treatment initiation. We also obtained electrocardiograms and chest roentgenograms and a questionnaire for parents about their lesion anxiety and treatment satisfaction (Fig. 1).

**Primary outcome measures**

The primary efficacy endpoint was the lesion redness percent change. The lesion redness was measured digitally as described below. The lesions showed a red to bluish-purple color. We resolved the color images into RGB images (red, blue, and green) and obtained fewer green lights than the surrounding healthy skin. We used this phenomenon to evaluate the color tone of the lesion changing to approach the healthy skin tone. We converted the 8-bit TIFF images into RGB images using Image J (free software for image analysis, https://imagej.softonic.jp/), before replacing each color image with a gray scale image of 256 gradations. We calculated the average image brightness value of the lesion in the green image using Image J, and both the percent change and the difference in the lesion redness in each case as per the formulas:

\[ Dif = Vb - Va \]
\[ Per = (Vb - Va) / Vb, \]

where \( Dif \) is the difference in lesion redness in each case, \( Per \) is the percent change in the lesion redness in each case, \( Vb \) is the average image brightness value of the lesion before treatment in each case and \( Va \) is the average image brightness value of the lesion after treatment in each case.
Secondary outcome measures

The secondary efficacy endpoints were lesion area (long axis × short axis in mm), a change in the perceived psychological anxiety against the lesion, the parents’ treatment satisfaction, the frequency of external treatment applications and treatment safety. We used questionnaires scored on a 6-grade scale to assess psychological anxiety and on a 5-grade scale to assess parents’ treatment satisfaction (Fig. 1). The questionnaire was created in consideration of the following points: using relatively easy sentences; expressing the emotions of parents with facial illustrations and filling out the questionnaire in a short time with few questions. The secondary safety endpoints included changes in skin characteristics, symptoms, objective findings, vital signs, and laboratory tests.

Sample size

The null hypothesis stated the absence of differences in the lesion redness percent change between the groups. Based on the results of dye laser treatments in 24 patients with port wine stain, we assumed an expected difference in proportion of 0.78% between the groups and a standard division of 0.36 (Table 1). Based on these assumptions, the required sample size was 6 per group (2-sided, α = 0.025 [two comparisons], β = 0.2, two-sample t-test) under the conditions of an assignment ratio of 1:1:1.

Statistical evaluation

We performed primary analyses of efficacy and safety on both the excluded population with serious test protocol violations (per-protocol set; PPS) and the population of patients who had undergone the respective study-specified assessments at least once after the start of study treatment (full analysis set; FAS). We made two comparisons to evaluate the superiority of 1% and 5% propranolol gels against the placebo gel for a primary endpoint analysis. We used a two-sample t-test for analysis and set the significance level per comparison at 2.5% to consider multiplicity by Bonferroni's method. Therefore, the significance level for the whole study was 5%.

To supplement the analysis of the primary endpoints, we analyzed the secondary endpoints of the efficacy. We used the t-test to compare the lesion areas, the change in psychological burden due to the lesion and the degree of treatment satisfaction. We calculated summary statistics (means and standard deviations, medians, and ranges) for each group, and the 95% confidence intervals of the mean for continuous values.

We recorded the frequency and proportion of adverse events and created a list of these and calculated the frequency of side effects. We performed all statistical analyses using the SAS statistical software package, version 9.4 (SAS Institute, NC, USA).
Ethical Approval

We conducted this study in accordance with the tenets of the Declaration of Helsinki and good clinical practice guidelines. The review board of Clinical Research Center, Chiba University Hospital approved the study (approval no. CRB3180015), and all patients or their parents provided written informed consent before their participation in the study.

Clinical trial registration

The study information is registered with UMIN Clinical Trials Registry, number UMIN000017148 and with Japan Registry of Clinical Trials, number jRCTs031180234.

Results

Patients

Of the 19 patients who provided written informed consents, all of them received the treatment. Overall, 17 patients completed the study and 15 were included in the PPS (Table 2 and Fig. 2). We found other similar baseline patient medical histories (laser irradiation, previous propranolol medication and timolol ointment) in the efficacy assessments among the treatment groups.

Efficacy

In the PPS, at the primary endpoint, the lesion redness percentage changes of each group were the same (i.e., 0.9; Table 3). However, in the FAS, the difference in tumor brightness in the RGB image suggested a slight trend for decreasing brightness as the propranolol concentration went up (Table S). Representative photographs of patients treated with 0%, 1% or 5% propranolol gels show macroscopic improvement (Fig. 3).

In the PPS and FAS, at the secondary endpoint, the lesion area was not reduced in size.

In the PPS, at the secondary endpoint, we observed a decrease in the psychological anxiety due to the lesion in the parents of patient treated with 5% propranolol. In the FAS this tendency was emphasized, but did not reach statistical significance (Table 4 and Fig. 4).

In the PPS, at the secondary endpoint, the treatment satisfaction of parents was high in the patients treated with 5% propranolol gel (Table 5). The difference between the placebo and 5% propranolol treated groups reached significance (p = 0.08). There seemed to be meaningful differences, but the differences were not statistically significant. In 2 of the 19 patients, redness relapse was observed.
during the follow-up period after the end of the treatment. Both patients were treated with 5% propranolol gel.

In the PPS, at the secondary endpoint, the frequency of external treatment applications was similarly good in the three groups.

Safety and tolerability
No serious adverse events were encountered in patients that would require the discontinuation of the gel, such as hypotension, hypoglycemia or skin necrosis. Overall, itching at the topical areas was reported in 4 of the 19 patients (21.1%; Table 6). One patient receiving 1% gel treatment developed asthma during the study period and we had to exclude him from the study owing to his asthma treatment.

Discussion
This single-center, double-blind, randomized, placebo-controlled trial found no significant differences in lesion redness reduction among the three groups. Some reports indicate that topical β-blocker treatment reduces IH. However, the findings of our study did not support this conclusion. We propose three factors that may have contributed to these clinical outcomes. First, topical medications may be wiped off with clothes before they are adequately absorbed into the skin. We measured the concentration of timolol and propranolol released from the gel formulation using cellulose membrane in a stationary Franz cell. The concentration of medicinal ingredients increased linearly for the first hour. No further increase in concentration was observed after 2 hours.
Second, the redness of the lesions may be reduced by moisturizing the affected area with the placebo gel. Dry dermatitis may contribute to the redness of IH after the proliferative phase. Desquamation is often present on the surface of IH in babies and moisturizing hemangiomas may help prevent ulceration. Third, a thin film sometimes forms after gel coating. Gels applied thickly onto a lesion may form a thin film that adheres to the skin. If a child scratches this area to remove the film, the redness of the lesion may be exacerbated. In this study, we used HM-HPMC as the gel base because gel preparations can be made easily with HM-HPMC and HM-HPMC-based gels have a higher viscosity and show plasticity with lower polymer concentrations than HPMC-based gels. These characteristics aid in providing good malleability and a reduction in the amount of base that remains once the gel has dried. Although this was expected to improve patient adherence, four patients (21.1%) complained of itching. Improved substrates for topical use in infants should be the subject of future research.
In gel form, the dosage of the drug may be reduced to ≤1/20 that of oral treatment. Thus, topical medication may reduce the risk of serious adverse events such as hypoglycemia, hypotension,
bradycardia, or skin atrophy.\textsuperscript{(9,20-23,30)} We observed no serious adverse events in our patients. One patient in the group using 1% propranolol gel developed asthma during the study period and the condition persisted after discontinuing the gel. A causal association between the onset of asthma and the use of 1% propranolol gel is unlikely. The lack of serious side effects is important to note for future investigations.

There were insufficient study subjects who met the study criteria and study protocol without serious violations. In the larger study group, the PPS, there were 15 study subjects before topical treatments (Table 2), which is already less than the calculated required sample size of 18 for the study. Despite an already insufficient sample size based on calculations, 15 study subjects before treatment were further reduced to only 8 after topical treatments (Tables 3, 4, 5, and 6 and Fig. 2). Unfortunately, we were underpowered to answer the question of efficacy.

Another major limitation of the study is restriction to the involuting phase where self-resolution is occurring. It is difficult to distinguish between effects of topical medicine and self-resolution. It was hypothesized that if the three topical agents could produce concentration-dependent results, the effect of spontaneous involution could be corrected in setting the study design. Typically, IH is treated during the proliferative phase in clinical practice and beta-blocker oral treatment is very effective against glowing IH. Owing to ethical considerations, we had adopted a study design in which the study subjects were in the involuting phase rather than the proliferative phase during which oral administration is permitted. We need different outcome measurement of treatment against IH in the proliferative phase. Because we have already experienced many laser treatments against port wine stains that do not worsen in a short period, we created and adopted a new method to judge the primary outcome based on photographs of port wine stains cases where the effect of laser irradiation was very high and cases where the effect was low (Table 1). And according to Principles for study subjects to be minimal we had concluded we were able to infer expected differences in 18 proportions, including some drop out cases.

Participant drop out because of steroid medication or loss to follow-up occurred in this study. The upper respiratory tract inflammation and eczema occurred frequently during the study. Additionally, it may be difficult to continue participating in the research due to birth of siblings. Drop-outs occurred from the specified small sample size, making the planned statistical evaluation difficult. We failed to detect an obvious dose-dependent effect for the propranolol gel treatment. If it is permissible to judge several results, including secondary outcome measures comprehensively, our results suggest the presence of redness reductions in the affected areas and the reduction in the parents’ anxiety toward the lesions (Tables S and 4 and Figs. 3 and 4). We also observed a marked redness relapse during the follow-up period in two cases that were treated with 5% propranolol gel. (We performed dye laser irradiation against the recurrence lesion.)
Those results may be caused by medication, self-care and by slow natural shrinking of involuting and/or involuted IH.\(^{3,4}\) The parents' satisfaction with propranolol gel treatment may reflect these results (Table 5). Twice-a-day external applications are less burdensome for parents and lead to good compliances within self-report. Of course, it cannot be said that the face illustrations accurately indicate parents’ complex emotions, including disappointment, worry, hope and peace. Moreover, our questionnaire was not designed to extract issues of topical treatment in practice.

Although previous timolol gel topical therapy was effective for small IH, our results suggest the presence of redness reductions in the affected areas with no clinical significance. However, our results imply that the topical propranolol gel has a limited effect on reducing the anxiety of parents and a favorable safety profile in Japanese pediatric patients with IH.

**Conflict of Interest**

The authors declare no conflict of interest.

**Supplementary Materials**

The online version of this article contains supplementary materials.
References

1) Tollefson MM, Frieden IJ. Early growth of infantile hemangiomas: what parents' photographs tell us. *Pediatrics*, **130**, e314–e320 (2012).

2) Chang LC, Haggstrom AN, Drolet BA Baselga E, Chamlin SL, Garzon MC. Growth characteristics of infantile hemangiomas: implications for management. *Pediatrics*, **122**, 360–367 (2008).

3) Krowchuk DP, Frieden IJ, Mancini AJ Darrow DH, Blei F, Greene AK. Clinical practice guideline for the management of infantile hemangiomas. *Pediatrics*, **143**, e20183475 (2019).

4) Harter N, Mancini AJ. Diagnosis and management of infantile hemangiomas in the neonate. *Pediatr. Clin. North. Am.*, **66**, 437–459 (2019).

5) Léauté-Labrèze C, Dumas de la Roque E, Hubiche T, Boralevi F, Thambo JB, Taïeb A. Propranolol for severe hemangiomas of infancy. *N. Engl. J. Med.*, **358**, 2649–2651 (2008).

6) Léaute-Labrèze C, Boccara O, Degrugillier-Chopinet C, Mazereeuw-Hautier J, Prey S, Lebbé G, Gautier S, Ortis V, Lafon M, Montagne A, Delarue A, Voisard JJ. Safety of oral propranolol for the treatment of infantile hemangioma: A systematic review. *Pediatrics*, **138**, e20160353 (2016).

7) Hoeger PH, Harper JI, Baselga E, Bonnet D, Boon LM, Ciofi Degli Atti M, El Hachem M, Oranje AP, Rubin AT, Weibel L, Léauté-Labrèze C. Treatment of infantile haemangiomas: recommendations of a European expert group. *Eur. J. Pediatr.*, **174**, 855–865 (2015).

8) Ostman-Smith I. Hypertrophic cardiomyopathy in childhood and adolescence - strategies to prevent sudden death. *Fundam. Clin. Pharmacol.*, **24**, 637–652 (2010).

9) Love JN, Sikka N. Are 1-2 tablets dangerous? Beta-blocker exposure in toddlers. *J Emerg. Med.*, **26**, 309–314 (2004).

10) Filippi L, Cavallaro G, Fiorini P, Daniotti M, Benedetti V, Cristofori G, Araiomo G, Ramenghi L, La Torre A, Fortunato P, Pollazzi L, la Marca G, Malvagia S, Bagnoli P, Ristori C, Dal Monte M, Bilja AR, Isacchi B, Furlanetto S, Tinelli F, Cioni G, Donzelli G, Osnaghi S, Mosca F. Study protocol: safety and efficacy of propranolol in newborns with retinopathy of prematurity (PROP-ROP): ISRCTN18523491. *BMC Pediatr.*, **10**: 83 (2010).

11) Püttgen K, Lucky A, Adams D, Pope E, McCuaig C, Powell J, Feigenbaum D, Savva Y, Baselga E, Holland K, Drolet B, Siegel D, Morel KD, Garzon MC, Mathes E, Lauren C, Nopper A, Horii K, Newell B, Song W, Frieden I; Hemangioma Investigator Group. Topical timolol maleate treatment of infantile hemangiomas. *Pediatrics*, **138**, e20160355 (2016).

12) Chan H, McKay C, Adams S Wargon O. RCT of timolol maleate gel for superficial infantile hemangiomas in 5- to 24-week-olds. *Pediatrics*, **131**, e1739–e1747 (2013).
13) Chakkittakandiyil A, Phillips R, Frieden IJ, Siegfried E, Lara-Corrales I, Lam J, Bergmann J, Bekhor P, Poorsattar S, Pope E. Timolol maleate 0.5% or 0.1% gel-forming solution for infantile hemangiomas: a retrospective, multicenter, cohort study. *Pediatr. Dermatol.*, **29**, 28–31 (2012).

14) Chambers CB, Katowitz WR, Katowitz JA, Binenbaum G. A controlled study of topical 0.25% timolol maleate gel for the treatment of cutaneous infantile capillary hemangiomas. *Ophthalmic Plast. Reconstr. Surg.*, **28**, 103–106 (2012).

15) Danarti R, Ariwibowo L, Radiono S, Budiyanto A. Topical timolol maleate 0.5% for infantile hemangioma: Its effectiveness compared to ultrapotent topical corticosteroids - A single-center experience of 278 cases. *Dermatology*, **232**, 566–571 (2016).

16) Yu L, Li S, Su B, Liu Z, Fang J, Zhu L, Huang M, Shan W, Song D, Ye B, Luo C. Treatment of superficial infantile hemangiomas with timolol: Evaluation of short-term efficacy and safety in infants. *Exp. Ther. Med.*, **6**, 388–390 (2013).

17) Oranje AP, Janmohamed SR, Madern GC, de Laat PC. Treatment of small superficial haemangioma with timolol 0.5% ophthalmic solution: a series of 20 cases. *Dermatology*, **223**, 330–334 (2011).

18) Moehrle M, Léauté-Labrèze C, Schmidt V, Röcken M, Poets CF, Goelz R. Topical timolol for small hemangiomas of infancy. *Pediatr. Dermatol.*, **30**, 245–249 (2013).

19) Ni N, Guo S, Langer P. Current concepts in the management of periocular infantile (capillary) hemangioma. *Curr. Opin. Ophthalmol.*, **22**, 419–425 (2011).

20) Price A, Rai S, Mcleod RWJ, Birchall JC, Elhassan HA. Topical propranolol for infantile haemangiomas: a systematic review. *J. Eur. Acad. Dermatol. Venereol.*, **32**, 2083–2089 (2018).

21) Ovadia SA, Landy DC, Cohen ER, Yang EY, Thaller SR. Local administration of β-blockers for infantile hemangiomas: a systematic review and meta-analysis. *Ann. Plast. Surg.*, **74**, 256–262 (2015).

22) Mashiah J, Kutz A, Rabia SH, Ilan EB, Goldberg I, Sprecher E, Harel A. Assessment of the effectiveness of topical propranolol 4% gel for infantile hemangiomas. *Int. J. Dermatol.*, **56**, 148–153 (2017).

23) Xu G, Lv R, Zhao Z, Huo R. Topical propranolol for treatment of superficial infantile hemangiomas. *J. Am. Acad. Dermatol.*, **67**, 1210–1213 (2012).

24) Takatsuka H, Suzuki T, Yamazaki S, Suzuki T, Rikihisa N, Mitsukawa N, Ishii I. Pharmaceutical evaluation of the hospital grade preparation of timolol gel for infantile hemangioma. *J. Pharm. Health. Care. Sci.*, **43**, 706–712 (2017) (in Japanese).

25) Buchanan H, Niven N. Validation of a facial image scale to assess child dental anxiety. *Int. J. Paediatr. Dent.*, **12**, 47–52 (2002).
26) Fay A, Nguyen J, Waner M. Conceptual approach to the management of infantile hemangiomas. *J. Pediatr.*, **157**, 881–888 (2010).

27) O TM, Scheuermann-Poley C, Tan M, Waner M. Distribution, clinical characteristics, and surgical treatment of lip infantile hemangiomas. *JAMA Facial. Plast. Surg.*, **15**, 292–304 (2013).

28) Ikeda K, Saitoh I, Oguma T, Takagishi Y. Effect of Hydrophobically modified hydropropyl methylcellulose on the crystallization from supersaturated solutions of indomethacin. *Chem. Pharm. Bull.*, **42**, 2320–2326 (1994).

29) Ghosal K, Chandra A, Rajabalaya R, Chakraborty S, Nanda A. Mathematical modeling of drug release profiles for modified hydrophobic HPMC based gels. *Pharmazie.*, **67**, 147–155 (2012).

30) Novoa M, Baselga E, Beltran S, Giraldo L, Shahbaz A, Pardo-Hernandez H, Arevalo-Rodriguez I. Interventions for infantile haemangiomas of the skin: abridged Cochrane systematic review and GRADE assessments. *Br. J. Dermatol.*, **180**, 527–533 (2019).
1. Please select from the picture below the figure that best reflects your feelings when looking at the affected area (infantile hemangioma) during the past month.

☑ Check the box that applies best.

Score

☐ 0  ☐ 1  ☐ 2  ☐ 3  ☐ 4  ☐ 5

2. How satisfied were you with this treatment overall?

☑ Check the box that applies best.

| Score          |
|----------------|
| Very satisfied |
| Satisfied      |
| Somewhat satisfied |
| Slightly satisfied |
| Not at all satisfied |

Figure 1. Questionnaires filled out by patients’ parents before and after the treatment.
Figure 2. Consort flow diagram.
Figure 3. Comparison of outcomes between 0% (placebo), 1%, and 5% propranolol gel treatments.

We photographed the tumours with a seal for colour correction before and after the treatment. The brightness value is the average of the lesion brightness in a grey scale image converted from a green RGB colour model image.

3a) Representative patient (Code No. 016) results after placebo treatment.

Left: Brightness value before treatment, 153.
Right: Brightness value after treatment, 144.

Percentage change in lesion redness (brightness value), 0.1%.
Difference in lesion redness (brightness value), 9.
3b) Representative patient (Code No. 007) results after treatment with 1% propranolol.

Left: Brightness value before treatment, 183.

Right: Brightness value after treatment, 123.

Percentage change in lesion redness (brightness value), 33%.

Difference in lesion redness (brightness value), 60.
3c) Representative patient (Code No. 017) results after treatment with 5% propranolol.

Left: Brightness value before treatment, 131.
Right: Brightness value after treatment, 94.

Percentage change in lesion redness (brightness value), 28%.
Difference in lesion redness (brightness value), 37.
Figure 4. Psychological anxiety changes before and after lesion treatment in the full set analysis.

The vertical bar length indicates the 95% confidence interval in the treatment group.
The centre mark indicates the mean value of scores in the treatment group.
No significant differences were found in the mean score changes between groups.
Table 1. Change in lesion redness in 24 patients with port wine stain

| Laser irradiation | Before treatment | After treatment | Dif    | Per    |
|-------------------|------------------|----------------|--------|--------|
| Effective cases   | Average brightness value | Average brightness value |        |        |
| 1                 | 123.29           | 81.80          | 41.49  | 3.365 × 10^1 |
| 2                 | 199.39           | 133.12         | 66.27  | 3.3236 × 10^1 |
| 3                 | 186.92           | 130.49         | 56.43  | 3.0189 × 10^1 |
| 4                 | 186.99           | 105.63         | 81.36  | 4.3510 × 10^1 |
| 5                 | 151.35           | 98.11          | 53.24  | 3.5177 × 10^1 |
| 6                 | 167.12           | 112.48         | 54.64  | 3.2695 × 10^1 |
| 7                 | 164.13           | 81.53          | 82.60  | 5.033 × 10^1 |
| 8                 | 193.54           | 79.87          | 113.67 | 5.873 × 10^1 |
| 9                 | 137.53           | 91.28          | 46.25  | 3.363 × 10^1 |
| 10                | 112.49           | 68.61          | 43.88  | 3.901 × 10^1 |
| 11                | 216.81           | 162.20         | 54.612 | 2.5189 × 10^1 |
| 12                | 162.66           | 100.55         | 62.11  | 3.8184 × 10^1 |
| Ineffective cases |                  |                |        |        |
| 1                 | 188.26           | 178.45         | 9.81   | 5.2109 × 10^2 |
| 2                 | 185.14           | 154.18         | 30.96  | 1.6722 × 10^1 |
| 3                 | 200.67           | 182.83         | 17.84  | 8.8902 × 10^2 |
| 4                 | 128.34           | 114.18         | 14.16  | 1.1033 × 10^1 |
| 5                 | 171.49           | 185.94         | −14.45 | −8.4261 × 10^2 |
| 6                 | 175.27           | 167.79         | 7.48   | 4.2677 × 10^2 |
| 7                 | 84.59            | 95.97          | −11.38 | −1.345 × 10^1 |
| 8                 | 84.59            | 71.14          | 13.45  | 1.590 × 10^1 |
| 9                 | 168.94           | 194.34         | −25.40 | −1.5035 × 10^1 |
| 10                | 151.7            | 135.73         | 15.97  | 1.0527 × 10^1 |
| 11                | 162.84           | 147.00         | 15.84  | 9.7273 × 10^2 |
| 12                | 179.18           | 194.975        | −15.80 | −8.8152 × 10^2 |

The average image brightness value of the lesion in the green image was calculated using Image J software. Dif, difference in lesion redness; Per, percent change in lesion redness.
Table 2. Demographics of the patients in per-protocol set (PPS)

| Variables                        | Total (n = 15) | Placebo group (n = 7) | 1% Group (n = 5) | 5% Group (n = 3) | p-value |
|----------------------------------|----------------|-----------------------|------------------|-----------------|---------|
| Age (y), mean (SD)               | 3.2 (1.8)      | 3.1 (2.4)             | 3.5 (1.2)        | 3.0 (2.8)       | 0.871   |
| Sex, Male/Female                 | 2/13           | 1/6                   | 1/4              | 0/3             | 1.00    |
| Height (cm), mean (SD)           | 91.1 (13.8)    | 91.6 (18.7)           | 92.1 (10.7)      | 88.4 (7.0)      | 0.940   |
| Weight (kg), mean (SD)           | 14.5 (7.6)     | 15.8 (11.1)           | 12.9 (2.9)       | 14.1 (2.0)      | 0.835   |
| Gestational age (week), mean (SD)| 38.2 (1.9)     | 39.7 (0.8)            | 36.5 (1.0)       | 37.0 (1.7)      | 0.001   |
| Birth weight (kg), mean (SD)     | 27322.2 (520.2)| 3018.6 (259.1)        | 2300.0 (550.6)   | 2784.3 (573.2)  | 0.047   |

Medical history

|                      | + | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
|----------------------|---|--------|--------|--------|--------|
| Asthma, n (%)        |   | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| Heart disease, n (%) | + | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
|                      |   | 15 (100%) | 7 (100%) | 5 (100%) | 3 (100%) |
| Others, n (%)        | + | 2 (6.7%) | 1 (14.3%) | 0 (0%) | 0 (0%) |
|                      |   | 13 (93.3%) | 6 (85.7%) | 5 (100%) | 3 (100%) |

n, number of patients. p-value, significance probability.
Table 3. Primary endpoint: Percentage change in lesion redness in per-protocol set (PPS)

| Visit                      | Group              | n  | Average       | Standard deviation | Median | Range | *Average difference | 95% CI of the difference | p-value | p-value after adjustment |
|----------------------------|--------------------|----|---------------|--------------------|--------|-------|--------------------|--------------------------|---------|--------------------------|
| Before topical treatment   | Placebo            | 3  | 145.4         | 30.8               | 139.9  | 88.4  |                   |                          |         |                          |
|                            | 1% Propranolol     | 3  | 132.8         | 52.4               | 157.3  | 127.4 |                   |                          |         |                          |
|                            | 5% Propranolol     | 2  | 138.0         | 17.1               | 129.4  | 30.9  |                   |                          |         |                          |
| After topical treatment    | Placebo            | 3  | 174.9         | 27.9               | 181.0  | 54.8  |                   |                          |         |                          |
|                            | 1% Propranolol     | 3  | 121.3         | 4.3                | 123.5  | 7.6   |                   |                          |         |                          |
|                            | 5% Propranolol     | 2  | 156.1         | 6.9                | 156.1  | 9.8   |                   |                          |         |                          |
| Rate of changes (i.e., P)  | Placebo            | 3  | 0.9           | 0.2                | 0.9    | 0.4   |                   |                          |         |                          |
|                            | 1% Propranolol     | 3  | 0.9           | 0.5                | 0.9    | 1.0   | 0.04              | (−0.69, 0.78)            | 0.88    | 1.00                     |
|                            | 5% Propranolol     | 2  | 0.9           | 0.1                | 0.9    | 0.1   | 0.01              | (−0.81, 0.84)            | 0.97    | 1.00                     |

*Each group vs. placebo group. n, number of patients. CI, confidence interval. p-value, significance probability.
### Table 4. Secondary endpoint: Lesion anxiety changes in the per-protocol set (PPS)

| Difference in changes | Group            | n  | Average value | Median | Range | *Average difference | 95% CI of the difference | p-value |
|-----------------------|------------------|----|----------------|--------|-------|---------------------|--------------------------|---------|
|                       | Placebo          | 3  | 0.7            | 0.0    | 4.0   |                     |                          |         |
|                       | 1% Propranolol   | 3  | 0.0            | 0.0    | 4.0   | −0.7                | (−4.7, 3.4)              | 0.69    |
|                       | 5% Propranolol   | 2  | −1.0           | −1.0   | 2.0   | −1.7                | (−6.2, 2.9)              | 0.39    |

Analysis by $t$-test; not adjusted for multiplicity. *Each group vs. placebo group. n, number of patients. CI, confidence interval. $p$-value, significance probability.
Table 5. Secondary endpoint: Parent satisfaction with treatment in the per-protocol set (PPS)

Analysis by $t$-test. We did not adjust for multiplicity. *Each group vs. placebo group. n, number of patients. CI, confidence interval. $p$-value, significance probability.

| Group            | n   | Average value | Median | Range | *Average difference | 95% CI of the difference | p-value |
|------------------|-----|---------------|--------|-------|----------------------|--------------------------|---------|
| Placebo          | 3   | 3.7           | 4.0    | 1.0   | N                    | N                        |         |
| 1% propranolol   | 3   | 2.3           | 2.0    | 1.0   | −1.3                 | (−3.0, 0.4)              | 0.10    |
| 5% propranolol   | 2   | 2.0           | 2.0    | 2.0   | −1.7                 | (−3.6, 0.2)              | 0.08    |
Table 6. Secondary endpoint: Safety evaluation in the full analysis set

|                        | Entire study population | Placebo   | 1% Propranolol | 5% Propranolol |
|------------------------|-------------------------|-----------|----------------|----------------|
|                        | n = 19                  | n = 7     | n = 6          | n = 6          |
| Hypotension            | 0                       | 0         | 0              | 0              |
| Hypoglycemia           | 0                       | 0         | 0              | 0              |
| Asthma                 | 1 (5.3%)                | 0         | 1              | 0              |
| Contact dermatitis     | 0                       | 0         | 0              | 0              |
| Itch                   | 4 (21.1%)               | 1 (14.3%) | 1 (16.7%)      | 2 (33.3%)      |
| Pigmentation           | 0                       | 0         | 0              | 0              |
| Depigmentation         | 0                       | 0         | 0              | 0              |
| Skin atrophy           | 0                       | 0         | 0              | 0              |
| Other adverse events*  | 10 (52.2%)              | 5 (71.4%) | 2 (33.3%)      | 3 (50.0%)      |

*Acute upper respiratory inflammation, tympanitis, acute gastroenteritis and widespread eczema
n, number of patients.