Journal of Dermatological Treatment

ISSN: 0954-6634 (Print) 1471-1753 (Online) Journal homepage: https://www.tandfonline.com/loi/ijdt20

Fractional 2940-nm Er:YAG Laser-Assisted Drug Delivery of Timolol Maleate for the Treatment of Deep Infantile Hemangioma

Li Sun, Chenxia Wang, Yuting Cao, Xinxiang Lv, Limin Tian, Dandan Liu, Lizhong Li & Wenchao Zhao

To cite this article: Li Sun, Chenxia Wang, Yuting Cao, Xinxiang Lv, Limin Tian, Dandan Liu, Lizhong Li & Wenchao Zhao (2020): Fractional 2940-nm Er:YAG Laser-Assisted Drug Delivery of Timolol Maleate for the Treatment of Deep Infantile Hemangioma, Journal of Dermatological Treatment, DOI: 10.1080/09546634.2020.1729330

To link to this article: https://doi.org/10.1080/09546634.2020.1729330

Accepted author version posted online: 11 Feb 2020.

Submit your article to this journal

Article views: 9

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=ijdt20

Check for updates

Fractional 2940-nm Er:YAG Laser-Assisted Drug Delivery of Timolol Maleate for the Treatment of Deep Infantile Hemangioma

Running title: Laser-Assisted Drug Treatment IHs

Li Sun[1¶*], Chenxia Wang[2¶] , Yuting Cao[3¶] , Xinxiang Lv[1], Limin Tian[1], Dandan Liu[3], Lizhong Li[4], Wenchao Zhao[5]

[1]Department of Dermatology, Affiliated Hospital of Inner Mongolia Medical University, Hohhot, China.

[2]Department of Dermatology, Yulin No.2 Hospital, Shanxi Province, China

[3]Department of Dermatology, Inner Mongolia Maternal and Child Health Care Hospital, Hohhot, China.

[4]Maternal and Child Health Care and Family Planning Service Center, Qingshui River, Hohhot, Inner Mongolia, China

[5]Blood Center of Inner Mongolia, Hohhot, China

[¶]These authors contributed equally to this work.

[*]Corresponding author

Li Sun

Department of Dermatology, Affiliated Hospital of Inner Mongolia Medical University, Hohhot, China

Accepted Manuscript

E-mail: sunnyhong2020@126.com

Tel: +86-18047166628

Fax: +86-21-64085875

Abstract

Objective: To study the efficacy and safety of fractional 2940nm laser-assisted drug delivery of timolol maleate 0.5% solution for the treatment of deep IHs.

Methods: In this study, thirty deep IHs were included, and a fractional 2940nm laser was applied with 2-weeks intervals. Topical timolol maleate 0.5% was applied under occlusion for 30 minutes four times/day for 24 weeks. The plasma concentration of timolol maleate was monitored for 0.5h after the first treatment. The HAS scores and the depth, lateral diameter, vertical diameter of hemangiomas were evaluated before treatment, at 8 weeks and 24 weeks of treatment, and at 4 weeks after treatment.

Results: Twenty-three patients (76.7%) demonstrated excellent regression, 4(13.3%) showed good response, and 3(10%) experienced moderate regression. The HAS score was declined from 3.6±0.7 to 2.3±0.6 at 8 weeks, and from 1.3±0.5 to 0.8±0.5 at 24 weeks($P<0.05$). Plasma timolol concentration was not detected in 11 infants, and the rest ranged from 1.580pg/ml to 14.718pg/ml, which were <1ng/ml. No systemic complications were observed in any patients.

Conclusion Fractional 2940nm laser-assisted drug delivery of timolol maleate 0.5% is considered to be an effective and safe method for treating deep IHs.

Accepted Manuscript

Key Words: Fractional laser, Timolol maleate, Drug delivery, Deep IHs

Introduction

Infantile hemangiomas (IH) are the most common benign vascular tumors with a reported incidence rate of 4-10%[1, 2], and usually appears after several days to several weeks after birth. The initial manifestations of IH include congestive, telangiectatic patches[3]. Consecutive photographs of children showed the fastest growth of IH between 5.5 weeks and 7.5 weeks after birth[4], reaching 80% of the final volume by 3 months[5]. After that, there is a slow proliferating period from 6-9 months, which then gradually fades away after several years [6]. IHs are classified into superficial, deep and mixed according to the depth of the tumor[7]. Oral propranolol is the first-line treatment for deep IH[3], but is associated with adverse reactions such as hypoglycemia, low blood pressure, slow heart rate, bronchial spasm, wheezing, temporary dyspnea, drowsiness, etc in children[8]. In recent years, topical beta blockers were used for treating superficial and deep hemangiomas. Timolol is a non-selective hydrophilic beta blocker[8], and topical treatment of it effectively reduces systemic adverse reactions. Because of the barrier effect of the skin and the hydrophilicity of timolol, the percutaneous penetration of the drug is affected. Hence, fractional 2940-nm Er:YAG laser was used for transdermal delivery of 0.5% timolol maleate eye drops to treat deep IHs in children and evaluate its effectiveness and safety.

Accepted Manuscript

Materials and methods

Normal information:

Inclusion criteria：

According to medical history, clinical manifestations, and color Doppler ultrasonography, 30 infants with deep skin hemangiomas (from birth till 6 months) were diagnosed. There were 8 males and 22 females, with a monthly age of 2±0.6 months. One case had deep hemangioma; 9 cases were first child, 21 cases were second child, 14 cases were delivered by spontaneous vaginal delivery, and 16 cases were delivered by cesarean section. The onset time was 16±6 days, with a rapid growth time of 1±0.7 months, and was untreated. Hemangioma sites included head in 5 cases, face in 10 cases, trunk in 9 cases, extremities in 5 cases, and perineum in 1 case.

Exclusion criteria:

Children with bradycardia (less than 80 beats/min), congenital heart disease, family history of asthma or asthma, hypoglycemia (<3.8 mmol/L), hypotension (systolic blood pressure <80 mmHg, diastolic blood pressure <50 mmHg), abnormal coagulation, preterm infants (gestational age <37 weeks), low-birth weight infants (birth weight <2500g), vitiligo or family history of vitiligo, and who are allergic to maleate timolol were excluded.

Complete vital signs (body temperature, respiration, heart rate, blood pressure), cardiopulmonary examination, 12-channel electrocardiogram, blood routine, liver function, renal function, blood glucose, coagulation, and hemangiomas with color Doppler ultrasound before undergoing initial treatment were recorded.

Accepted Manuscript

Methods

Treatment

The subject's parents or guardians signed the informed consent by photo use agreement, established a personal file, and took photos with a digital camera (70D, Canon, Japan). The 5% lidocaine cream (Beijing Ziguang Pharmaceuticals) was used to partially block the hemangiomas for 40 minutes and then disinfected with 2% iodophors thrice. The part of the laser device that touches the skin was disinfected with Zeerdrin once. Then the fractional 2940-nm Er:YAG Laser of Profile Work Platform of US Sicton Company emitting laser (micropore aperture: 430um, dot matrix coverage: 11%, solidification depth: 80-110um) was used. External bleeding was stopped by using a disposable sterile cotton swabs, and 3-5 drops of 0.5% timolol maleate eye drops (Wuhan Wujing Pharmaceutical Factory) were applied topically immediately after treatment. The surface of hemangioma was covered with external 6-layer sterile gauze, and the outermost layer was covered with plastic wrap for 1 hour. Treatment was repeated every 2 weeks. The children continued to receive external topical 0.5% timolol maleate eye drops for half-an-hour four times a day. Treatment was repeated for a duration of 24 weeks.

For mixed hemangiomas, V-beam 595nm pulse dye (Candela) laser (pulse width 10ms, energy 8-10.5J/cm2, spot size 7mm) was performed. Treatment was conducted once every 2 weeks, and external medical ice packs were used immediately after treatment for half-an-hour. After that, 0.5% Timolol maleate eye drops package were used, each packet was used for half-an-hour four times a day to reduce the surface tension of hemangioma, and to darken the

Accepted Manuscript

color (after 2 times of average treatment). The fractional 2940nm Er:YAG laser assisted drug delivery was then used. The total course of treatment was 24 weeks.

Experimental Methods

Preparation of Standards

The standard timarol maleate (United States selleck Biotechnology) of 9.04g was weighed using an electronic balance, and then added into the mobile phase (acetonitrile: 0.05% formic acid solution = 80:20) of a 10ml volumetric flask to prepare a mother liquor with a concentration of 0.904mg/ml. 1μl plus 1ml mobile phase in 1.5ml EP tube was mixed well, and then was sucked to mix thoroughly. After homogenization, the absorbed 100ul and 1ml of the mobile phase were added into a 1.5ml EP tube. The sample was set to a standard concentration gradient of 9.040pg/ml, 7.232pg/ml, 5.424pg/ml, 3.616pg/ml, 1.808pg/ml, and 0.904pg/ml.

Sample Preparation

Half-an-hour after the first treatment, 2 ml of venous blood was drawn, and then was centrifuged at 3,500 rpm to collect the plasma. Centrifugation at 3,500 rpm was repeated again and then stored in a refrigerator at -80°C. After collecting the plasm, it was thawed in a refrigerator at 4°C, and then 100μl of plasma was added to 10μl of 0.05% formic acid solution. After vortexing, 240μl of acetonitrile was added. The mixture was shaken for 2 minutes and allowed to stand for 5 minutes. The mixture was centrifuged at 12,000 rpm for 10 minutes at 4°C. Next, 60ul clear solution was added to 540ul mobile phase.

The prepared standard and sample was then tested. The mobile phase conditions were as follows: column temperature 40°C, tray temperature 8°C, flow rate 0.4 ml/min, A: 0.1%

Accepted Manuscript

formic acid solution, and B: 0.1% formic acid solution + acetonitrile. Mass spectrometry conditions were as follows: capillary 3.00 KV, source temperatures 100°C, desolvation temperatures at 400°C., Cone Gas 100 L/h, and desolvation gas of 800 L/h.

Efficacy assessment

Before each treatment, the children were photographed under the same light and positioned using a digital camera (70D, Canon, Japan). According to the scores proposed by Achauer[9], the treatment effects were divided into four grades based on the degree of improvement in volume, color, and texture of the tumor before and after treatment: poor (0-25%), moderate (26%-50%), good (51%-75%), excellent (76%-100%). The Hemangioma Activity Score (HAS) score was obtained by two dermatologists before treatment, at 8 weeks of treatment (4 treatments), at 24 weeks of treatment (12 treatments), and at 4 weeks after treatment[10]. The hemangioma depth, lateral diameter, and vertical diameter were measured by color Doppler ultrasonography with hemangiomas before treatment, at 8 weeks, at 24 weeks after the treatment, and at 4 weeks after treatment.

Parents at home should apply the topical package of timolol maleate eye water for half-an-hour after measuring the heart rate in children with hemangiomas. If drowsiness, difficulty breathing, sweating and other adverse reactions, and heart rate of lower than 80 times/minute[11] were observed, then topical application of timolol maleate eye drops should be stopped and immediately taken to the hospital.

Vital signs and blood glucose should be recorded and reviewed before each laser treatment. Twelve-channel ECG, liver and kidney function, blood glucose, and hemangiomas were reviewed with color Doppler ultrasound after every 4 laser treatments. Patients treated

Accepted Manuscript

with V-beam 595nm pulsed dye laser were observed for adverse reactions such as erythema, edema, blisters, scarring, hyperpigmentation, hypopigmentation, and scars at the site during laser treatment.

Statistical analysis

All data were analyzed using SPSS 16.0 statistical software. The measurement data were expressed as $\overline{x}\pm s$. Paired t-test was used to compare the means before and after treatment. The count data was expressed as rate. $P<0.05$ was considered to be statistically significant.

Results

1. A total of 30 patients were included in this study, in which 23 patients had excellent treatment response (76.7%), 4 patients had good treatment response (13.3%), and 3 patients had moderate treatment response (10%).

2. The HAS score was 3.6±0.7 before treatment, which was reduced to 2.3±0.6 at 8 weeks ($P<0.05$), reduced to 1.3±0.5 at 24 weeks ($P<0.05$), and decreased to 0.8±0.5 at 4 weeks after the last treatment ($P< 0.05$). There was a significant difference between 24 weeks of treatment and 8 weeks of treatment ($P<0.05$). Also a significant difference was observed between 4 weeks after treatment and 24 weeks of treatment ($P<0.05$), (Figure 1, Figure 2, Figure 3, Table 1).

3. According to the standard curve (Table 2, Figure 4), the equation was as follows: $Y = 7.89 + 62.72X$, $R= 0.99973$ (Y: peak area, X: drug plasma concentration, R: fitness). After measuring the peak area of the sample and substituting it into the equation, the concentration of timolol maleate in the plasma was calculated. The results showed that timolol maleate was

Accepted Manuscript

not detected in 11 plasma samples, which was lower than the detection limit of 0.904pg/ml. The remaining 8 patients had plasma concentrations ranging from 1.580 pg/ml to 14.718 pg/ml, and all were below 1 ng/ml (Table 3).

4. No children experienced heart rate, blood pressure, and blood glucose reduction during treatment. No children had liver and kidney dysfunction, difficulty breathing, lethargy, sweating, etc. Three children with mixed hemangiomas were given V-beam 595nm pulse dye for the first time. After laser treatment, erythema, edema, blisters, and scarring were observed. No signs of pigmentation, hypopigmentation, scars or other adverse reactions were observed after shedding.

Discussion

The International Society for Hemangiomas and Vascular Anomalies (ISSVA) classified IH into three types based on the depth of tumor invasion in 2014. Superficial hemangiomas had dermal papilla and appeared bright red, deep hemangiomas showed reticular dermis and subcutaneous tissue, and appeared blue, purple or skin color, and mixed hemangiomas had manifestations of both superficial and deep hemangiomas[7, 12]. Treatment to IHs is necessary to inhibit the regeneration of vascular endothelial cells, promote the regression of tumors, and reduce residual tumors, which included topical drugs, systemic drugs, local injections, lasers, and surgical procedures[13]. Léauté-labrèze et al. in 2008 have treated hemangiomas with congestive heart failure in 2 children and unexpectedly discovered the subsidence of hemangiomas[13]. This finding provoked many scholars to conduct clinical observations and basic research. Propranolol has been gradually substituted by

Accepted Manuscript

glucocorticoids and has become the first-line drug for the treatment of deep IHs[3]. At present, the internationally accepted and commonly used dose of oral propranolol for treating deep IH is 2mg/Kg•d, which is divided into three oral doses[11], while the recommended dose in our country is 1.5-2mg/Kg•d, which is divided into two oral doses[2]. Propranolol as a non-selective lipophilic beta-blocker induces systemic adverse reactions when hemangiomas are treated orally through blood-brain barrier. If children have contraindications to the use of beta-blockers, glucocorticoids can be used systemically, but oral glucocorticoid systemic adverse reactions cannot be ignored, such as high blood pressure, mania, induced or aggravated ulcers, gastroesophageal reflux, nausea, vomiting, hypothalamic-pituitary-adrenal axis inhibition, growth retardation, immunosuppression, Cushing-like appearance, weight gain, etc. During the treatment, height, weight, and blood pressure should be continuously monitored[6].

Timolol maleate is another non-selective hydrophilic β-adrenoceptor blocker that has recently been used for the treatment of superficial and deep hemangiomas locally. Xu DP[14] et al. have used localized treatment of topical maleate timolol for infantile superficial hemangiomas safely and effectively. Xue K[7] et al. have successfully treated two children with deep hemangiomas around the eye with topical 0.5% timolol maleate eye drops and showed improvement in the visual acuity. Painter SL[15] et al. also successfully treated 5 children with hemangiomas who had deep peripheral and potentially disabling risk of ocular bleomycin by topical 0.5% timolol maleate solution. Sorrell J[16] et al. have successfully used topical 0.5% timolol maleate gel locally for treating 3 proliferating small facial IHs. Although these are only case reports and lacked large sample data, the local topical drugs are

Accepted Manuscript

replacing systemic drugs to reduce systemic adverse reactions.

Due to skin barrier, only 1%-5% of topical drugs were transdermally absorbed[17], and timolol is a hydrophilic substance that increases the difficulty of percutaneous penetration, which in turn affects the therapeutic efficacy of the drug. Fractional 2940-nm Er:YAG Laser is an Ablative Fractional Laser (AFXL) that forms a microscopic treatment zone (MTZ) in the beam irradiation area, and forms a columnar microscopic structure on the skin tissue after laser irradiation. The epidermal necrotic degeneration (MENDs) of the epidermis, which is equivalent to the formation of many tiny pores, allows timolol maleate to cross the skin barrier and transmit directly to the dermis. Thus, it greatly increased the transdermal absorption of the drug, and guaranteed the efficacy. In addition, fractional 2940-nm Er:YAG Laser precisely controls the depth, and due to tiny thermal denaturation of the epidermis, necrosis and surrounding normal tissues, the treatment areas are quickly recovered after treatment[18]. Ma Gang[19] et al. have used fractional CO_2 laser to assist the delivery of timolol maleate at a dose of 0.5% to treat 9 infants with proliferative deep hemangiomas. The results showed an excellent therapeutic response in 4 patients (44.4%), good treatment response in 4 patients (44.4%), and moderate treatment response in 1 patient. Our study expanded the sample size based on this, and conducted cumulative treatment in 32 cases with deep proliferative phase IH. Of these, 2 cases did not adhere to treatment, and the remaining 30 cases were treated according to the course of treatment. Based on the scores of volume, color and texture of the tumor before and after treatment, 23 patients had excellent treatment response (76.7%), 4 patients had good treatment response (13.3%), and 3 patients had moderate treatment response (10%). Three patients had moderate reactions due to the

Accepted Manuscript

following factors: 1. tumor depth, in which the treatment remains more difficult for deeper hemangiomas; 2. age of the patient was >3 months at the time of treatment and rapid growth of hemangiomas occur at 2 months of age. During the period of 3 months of age, tumors can reach 80% of the final volume, increasing the difficulty of treatment. Of the 2 patients who did not adhere to the treatment, 1 patient had chosen a local hospital for treatment due to inconvenience of remote home interviews, and the other had chosen a private hospital, with no known specific treatment. Common complications of IH included ulceration, disfigurement, and dysfunction, and are prone to early proliferative phase. Although IH in 1 year olds begin to enter the remission phase, 25% to 69% of children have tumor regression that is untreated. Residual telangiectasia, loose skin, atrophy, erythema, and hypopigmentation were also observed[6]. Therefore, treatment of deep IHs was performed during the period of rapid growth, and at the same time prevented the formation of ulcers in proliferative hemangiomas and increased the risk of scar formation, especially in the facial and genital areas. Children have a lifetime psychological and physical effects.

The HAS score showed statistically significant differences between 8 weeks of treatment, 24 weeks of treatment, 4 weeks after the end of treatment, and 4 weeks before treatment. There were significant differences between 24 weeks of treatment and 8 weeks of treatment, and at 4 weeks after the end of the treatment and 24 weeks of treatment. Significant differences indicated increased efficacy with increased treatment period. During the course of treatment, there was 1 case with deep hemangioma at the tip of the nose, and most of these tumors were dissipated after receiving 10 treatments (about 9 months of age). The family members stopped the treatment on their own, and hemangiomas were recurred at

Accepted Manuscript

about 11 months of age. The patient again continued the treatment until 15 months of age, and the tumors were subsided. This gives us an inspiration that the treatment of IH should be continued until the hemangiomas are completely subsided or until the child is 12 months of age. In another case, the His in right breast were treated until 10 months of age, and the tumor has been subsided. The family discontinued the treatment and relapsed at about 11 months of age. The treatment is continued until 13 months of age. The development of lateral mammary glands was seen in the Maternal and Child Health Hospital of the Inner Mongolia Autonomous Region. The diet-induced bilateral hyperplasia of the breast did not respond to the treatment. Therefore, treatment should be continued till 14 months of age, until the tumor is subsided.

Hemangiomas were reviewed with color Doppler ultrasound after every 4 laser treatments. The tumor depth, lateral diameter, and vertical diameter were measured. With increased treatment duration, although the HAS score is decreasing, the eye color becomes lighter, and the palpable tumors becomes soft, and the values of depth, the lateral diameter, and the vertical diameter of the tumor as measured by color Doppler ultrasound showed no reduction in most of the children. The increase or decrease of the disease was not obvious and cannot be statistically processed. This might be due to the following factors: On one hand, the crying of the child does not cooperate with the examination, increasing the difficulty of measurement by imaging physician and human error occurs in each measurement; and on the other hand, with the development of the child, the tumor body also constantly increases. However, after carefully reading the color Doppler results of all patients, the blood flow signal showed a gradual change from a reticulocyte blood flow signal to a banded blood flow

Accepted Manuscript

signal, a spotted blood flow signal, and there was no significant difference even with the peripheral tissue blood flow signal. This finding not only verified the physiological effects of timolol on blood vessel contraction, angiogenesis inhibition, and apoptosis of vascular endothelial cells promotion[8], but also was consistent with decreased HAS score, a lighter color of the naked eye, and a softening of the accessible tumor. This further confirmed the effectiveness of the treatment.

In addition, by taking into account the increase in local drug absorption due to systemic adverse reactions, a high-performance liquid chromatography mass spectrometry was used to detect the plasma timolol concentrations in 19 patients. The remaining children were not allowed for detection by their families for drawing blood. The results showed that timolol maleate was not detected in 11 plasma samples, which was lower than the detection limit of 0.904 pg/ml, and the remaining 8 samples were in the range of 1.580 pg/ml to 14.718 pg/ml, which were far below 1 ng/mL. According to the previous reports, plasma timolol concentrations of below 1 ng/mL are physiologically insignificant[20], producing no theoretical adverse reactions. In clinical practice, 30 children were monitored for heart rate, blood pressure, blood glucose, liver and kidney function, etc. We also urged parents to count their heart rate at home for half-an-hour after treatment and record any signs of adverse reactions such as drowsiness, difficulty breathing, and sweating. No adverse reactions were observed. Both aspects fully demonstrated the safety of treatment.

During the course of treatment, we unexpectedly found that the hair roots at the treated site were clearly visible in 5 children with deep hemangiomas in the head. Hair analysis also confirmed that hair follicles were normal (hair shaft diameter and color were the same, and

Accepted Manuscript

each hair follicle unit had 1-3 hairs, no black spots, broken hair, or comma-like hair, presumably normal hair growth), which is an advantage of our treatment. The laser penetration depth of Nd:YAG 1064nm was up to 4-6mm, which was clinically used to treat deep vascular lesions. When the laser was applied on the affected area of the hair, the melanin in the hair follicle absorbs laser energy and is converted into heat, which is then transferred to the surrounding tissues. Destruction of adjacent hair follicle stem cells might result in complications of no hair growth, and the therapeutic effect is limited if the penetration depth is limited, and are prone to burns, left scars, and other adverse reactions during the treatment[18]. Topical anesthesia by lidocaine cream was used before treatment. During the treatment, the patient had no pain to slight pain. Compared with Nd:YAG 1064nm laser, the locally injected drugs and other painful deep hemangioma treatments have other advantages. We also observed that none of the 30 patients had scars, and even some patients had no texture changes and color differences when compared to normal surrounding tissues, which is important for children with facial hemangiomas.

The superficial part of the hybrid hemangiomas is rich in blood vessels due to the presence of dermal papillary layer. If the first treatment involves the use of a fractional laser, it causes bleeding and local infections. The V-beam 595 nm pulsed dye laser is close to the third absorption peak of the hemoglobin in the visible spectrum and can be selectively absorbed by hemoglobin in the blood vessels. The laser energy is converted into heat energy, which is then transmitted to the surrounding blood vessel wall, causing damage to the blood vessel wall[18]. The mixed hemangiomas were treated with pulsed laser to perform lattice laser assisted drug delivery, which in turn reduces local bleeding and the probability of

Accepted Manuscript

infection. Local erythema, blisters and other adverse reactions may occur during the treatment. After disposable sterilized syringe was used to collect the sputum (the sputum wall remained intact), the use of 6 layers of sterile gauze pad and saline wet dressing showed improvement, and no pigmentation was left in the children. Sedation, hypopigmentation, and scarring were not also observed.

In summary, the fractional 2940 nm Er:YAG laser assisted by 0.5% timolol maleate delivery can effectively treat infantile deep hemangiomas without systemic adverse reactions and is considered to be safe and effective for deep infant treatment. It is a choice of treatment for deep hemangiomas. In addition, compared with oral propranolol treatment, our study treatment was considered to be safer, and broadened the clinical indications of treatment, preterm infants, low birth weight children, etc. However, some areas still need improvement. Only few samples were included because our department is unable to monitor the general vital signs of infants without setting up an oral drug control group, and lacked long-term follow-up of patients to assess the treatment. Our study also lacked long-term effects and aesthetic improvements. This study not only provides a convenient medical treatment for children and their families, but also avoids adverse drug reactions and shortens the treatment cycle. It is worth promoting.

Accepted Manuscript

Acknowledgments

Not applicable

Funding details

This work was supported by the Scientific Research Program of Inner Mongolia Health and Family Planning Commission in 2017

Disclosure statement

The authors report no conflict of interest

Data availability statement

Data sharing is not applicable to this article as no new data were created or analysed in this study.

Accepted Manuscript

References

1. Kilcline C, Frieden IJ. Infantile hemangiomas: how common are they? A systematic review of the medical literature. Pediatr Dermatol. 2008; 25 (2): 168-173.

2. Kanada KN, Merin MR, Munden A, et al. A prospective study of cutaneous findings in newborns in the United States: correlation with race, ethnicity, and gestational status using updated classification and nomenclature. J Pediatr. 2012; 161 (2): 240-245.

3. Guidelines for Diagnosis and Treatment of Hemangiomas and Vascular Malformations (2016 Edition). Journal of Tissue Engineering and Reconstruction Surgery. 2016; 02:

63-93+97.

4. Tollefson MM, Frieden IJ. Early growth of infantile hemangiomas: what parents' photographs tell us. Pediatrics. 2012; 130 (2): e314-320.

5. Chang LC, Haggstrom AN, Drolet BA, et al. Growth characteristics of infantile hemangiomas: implications for management. Pediatrics. 2008; 122 (2): 360-367.

6. Luu M, Frieden IJ. Haemangioma: clinical course, complications and management. Br J Dermatol. 2013; 169 (1): 20-30.

7. Wassef M, Blei F, Adams D, et al. Vascular Anomalies Classification: Recommendations From the International Society for the Study of Vascular Anomalies. Pediatrics. 2015; 136 (1): e203-214.

8. Painter SL, Hildebrand GD. Review of topical beta blockers as treatment for infantile hemangiomas. Surv Ophthalmol. 2016; 61 (1): 51-58.

9. Achauer BM, Chang CJ, Vander Kam VM. Management of hemangioma of infancy: review of 245 patients. Plast Reconstr Surg. 1997; 99 (5): 1301-1308.

10. Janmohamed SR, de Waard-van der Spek FB, Madern GC, et al. Scoring the proliferative activity of haemangioma of infancy: the Haemangioma Activity Score (HAS). Clin Exp Dermatol. 2011; 36 (7): 715-723.

11. Rouhana HF, Fruge JH, Massengale WT. Beta blocker treatment for infantile hemangiomas. Dermatol Online J. 2015; 21 (7):

12. Xue K, Hildebrand GD. Deep periocular infantile capillary hemangiomas responding to topical application of timolol maleate, 0.5%, drops. JAMA Ophthalmol. 2013; 131 (9): 1246-1248.

Accepted Manuscript

13. Leaute-Labreze C, Dumas de la Roque E, Hubiche T, et al. Propranolol for severe hemangiomas of infancy. N Engl J Med. 2008; 358 (24): 2649-2651.

14. Xu DP, Cao RY, Tong S, et al. Topical timolol maleate for superficial infantile hemangiomas: an observational study. J Oral Maxillofac Surg. 2015; 73 (6): 1089-1094.

15. Painter SL, Hildebrand GD. Topical timolol maleate 0.5% solution for the management of deep periocular infantile hemangiomas. J AAPOS. 2016; 20 (2): 172-174 e171.

16. Sorrell J, Chamlin SL. Topical timolol 0.5% gel-forming solution for small deep facial infantile hemangiomas. Pediatr Dermatol. 2013; 30 (5): 592-594.

17. Nelson JS, McCullough JL, Glenn TC, et al. Mid-infrared laser ablation of stratum corneum enhances in vitro percutaneous transport of drugs. J Invest Dermatol. 1991; 97 (5): 874-879.

18. Xiang L, Zhou Z. Principles and techniques of laser skin treatment. People's Medical Publishing House. 2014: 26-27.

19. Ma G, Wu P, Lin X, et al. Fractional carbon dioxide laser-assisted drug delivery of topical timolol solution for the treatment of deep infantile hemangioma: a pilot study. Pediatr Dermatol. 2014; 31 (3): 286-291.

20. McMahon P, Oza V, Frieden IJ. Topical timolol for infantile hemangiomas: putting a note of caution in "cautiously optimistic". Pediatr Dermatol. 2012; 29 (1): 127-130.

Accepted Manuscript

Table 1 Paired t test before and after treatment with HAS scores

Before Treatment	After Treatment	t	p
3.6±0.7*	2.3±0.6**	8.202	<0.05
3.6±0.7*	1.3±0.5#	14.486	<0.05
3.6±0.7*	0.8±0.5##	15.782	<0.05
2.3±0.6**	1.3±0.5#	9.942	<0.05
1.3±0.5#	0.8±0.5##	6.012	<0.05

Note: * HAS score before treatment, ** HAS score at 8 weeks of treatment, # HAS score at 24 weeks after treatment, ##HAS score at 4 weeks after treatment.

Table 2 Standard product curve

Number	Concentration (pg/mL)	Peak area
1	0.904	65
2	1.808	125
3	3.616	231
4	5.424	345
5	7.232	462
6	9.040	577

Accepted Manuscript

Table 3 Plasma concentration of timolol maleate (unit: pg/ml)

Number	1	2	3	4	5	6	7	8	9	10
Peak area	0	120	0	118	107	0	434	0	261	0
Concentration	-	1.787	-	1.756	1.580	-	6.794	-	4.036	-
Number	11	12	13	14	15	16	17	18	19	20
Peak area	0	0	348	0	124	0	0	0	0	931
Concentration	-	-	5.423	-	1.851	-	-	-	-	14.718

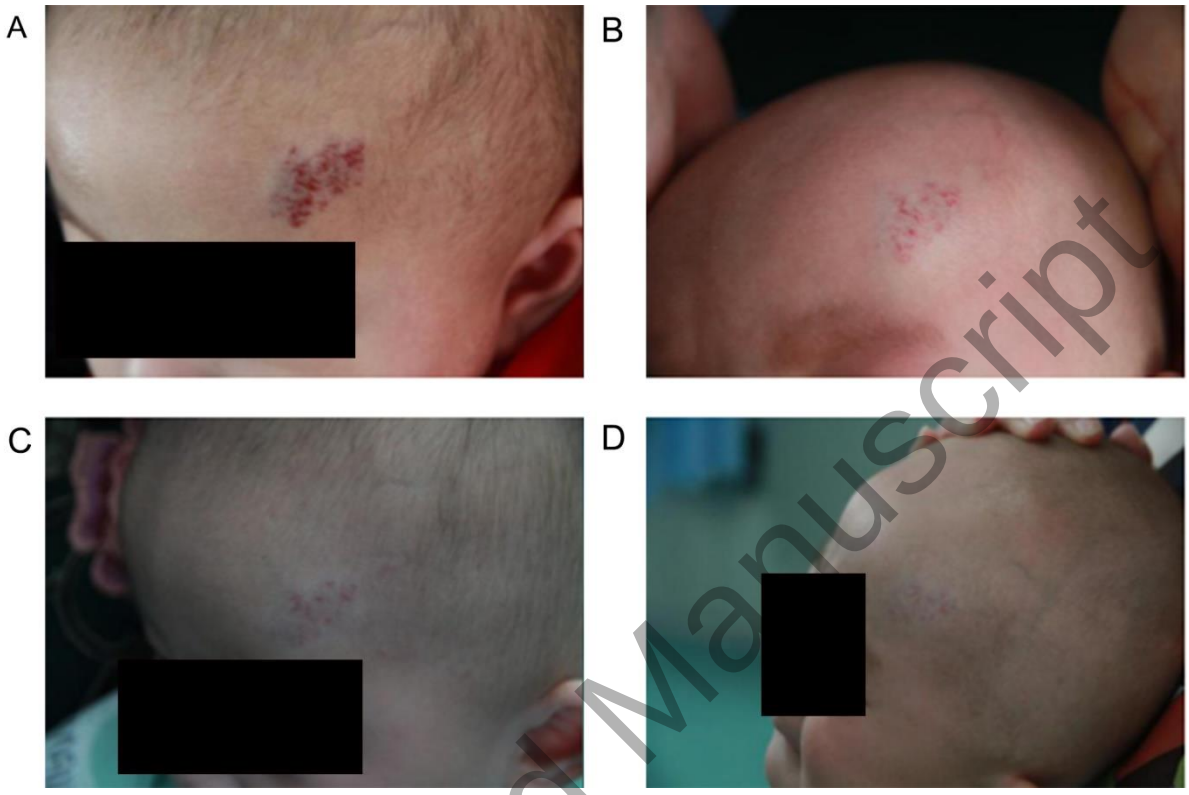
Note: 1 is a normal control group, 2-20 are case groups; a peak area of 0 meant that the timolol maleate concentration in plasma was lower than the detection limit of 0.904pg/ml.

Accepted Manuscript

Figure legends

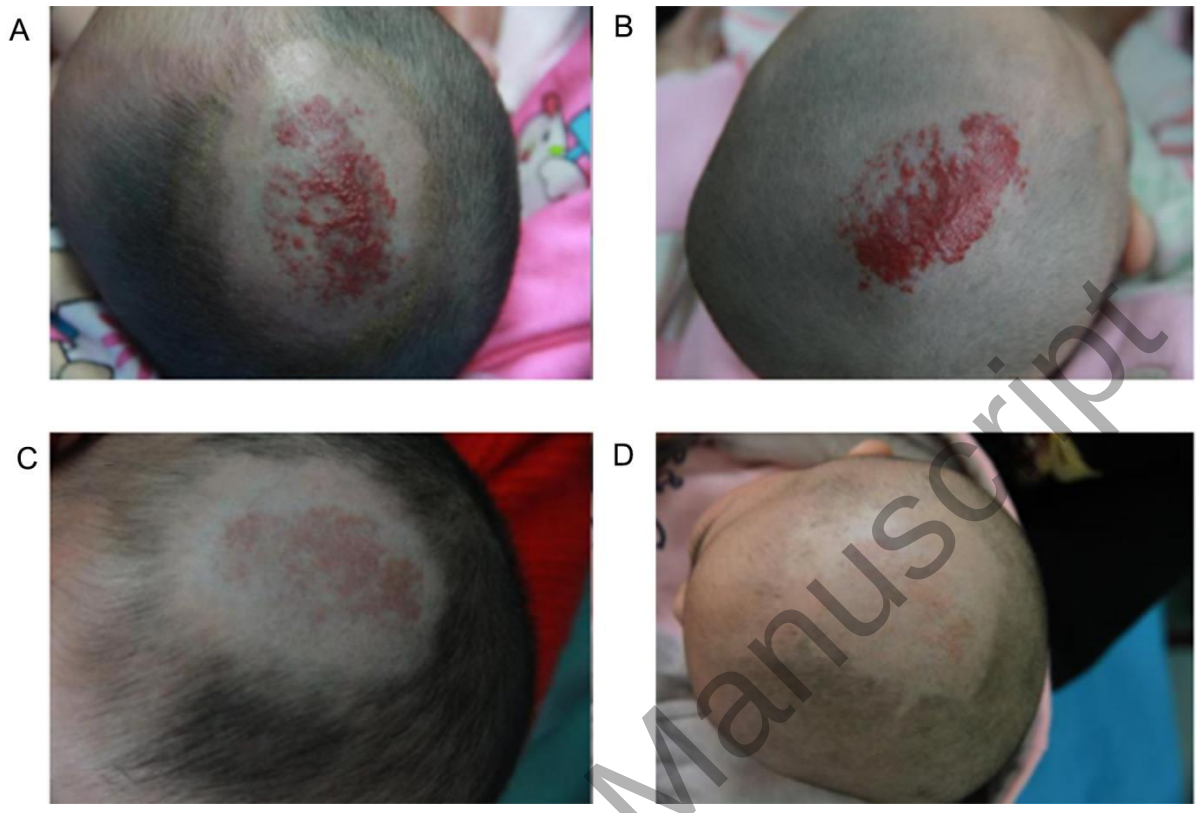
Figure 1 (A) Before treatment, HAS = 3; (B) 8 weeks of treatment, HAS = 2.3;

(C) HAS = 1.7 at 24 weeks of treatment; (D) HAS = 1.3 at 4 weeks after the end of treatment.



Accepted Manuscript

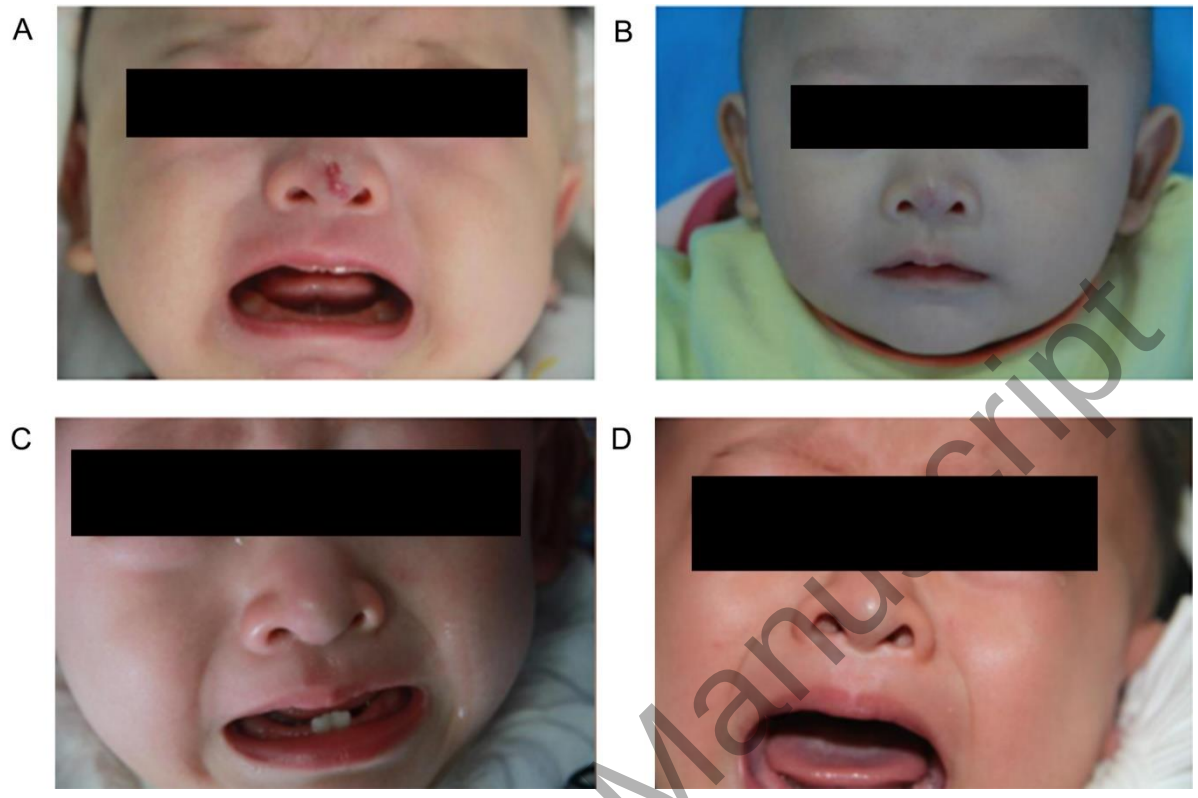
Figure 2 (A) Before treatment, HAS = 3.25; (B) 8 weeks of treatment, HAS = 3.25; (C) 24 weeks of treatment, HAS = 2.3; (D) HAS = 1.7 4 weeks after the end of treatment.



Accepted Manuscript

Figure 3 (A) Before treatment, HAS = 3.25; (B) 8 weeks of treatment, HAS = 2.3;

(C) Treatment for 24 weeks, HAS = 0.5; (D) HAS = 0.5 4 weeks after the end of treatment.



Accepted Manuscript

Figure 4 Standard product curve