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Efficacy of timolol in the treatment of facial hemangioma and its effect on the proliferation and apoptosis of hemangioma stem cells

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ABSTRACT

To investigate the efficacy of timolol in the treatment of facial hemangioma and the effect on the proliferation and apoptosis of hemangioma stem cells, 60 cases of children with IHs admitted to our hospital between 2020 and 2021 were selected and divided into two groups. The grouping was according to the lottery method, with 30 cases in each group. In the observation group, 0.5% timolol maleate eye drops were applied topically, and in the control group, propranolol hydrochloride tablets were administered orally to observe the efficacy of hemangioma, changes in hemangioma stem cells and the incidence of adverse reactions in both groups. Results showed that combined with the four-level score and ultrasound results, the number of effective treatment cases in the observation group was 28, which was higher than that in the control group, (P<0.05). The total number of adverse reactions in the observation group was 2, with an incidence rate. Under the intervention conditions of timolol, the proliferation level of hemangioma stem cells was inhibited, and the apoptosis rate of hemangioma stem cells increased with the increase of culture time (P<0.05). Among them, the apoptosis rate of the timolol group was higher than that of the blank control group at the same time point (P<0.05), and the difference was most significant at 48h (P<0.001). In conclusion, Timolol can effectively treat facial hemangioma in children, inhibit the proliferation of hemangioma stem cells and promote their apoptosis, with good curative effect, short treatment time and no obvious adverse reactions and it is economical and easy to accept.

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Introduction

Hemangioma is a congenital benign tumor or vascular malformation. The tumor area is bright red or purplish red irregularly distributed, protruding from the mucous membrane of the skin, soft and without pressure pain, and is most common on the face, lips and cheeks, and tongue. Hemangioma can destroy soft tissues and hard bone tissues in the body, which often leads to tissue loss or deformity in the face and affects beauty (1). Most hemangiomas occur in infants and children. Infantile hemangiomas (IHs) are vascular proliferations that arise during the embryonic period and are characterized by abnormal proliferation of vascular endothelial cells. It is a benign tumor arising in the skin and soft tissues. The incidence of this tumor in the population is about 4% to 5%, and the trend is increasing year by year. Although there are various theories such as placenta theory, endothelial progenitor cell theory, angiogenesis imbalance theory, gene mutation theory, developmental zone defect theory and estrogen theory, the pathogenesis of hemangioma is not clear until now. The tumor growth slows down and the texture becomes softer, which indicates that the lesion gradually enters the regressive phase from the proliferative phase (2). After entering the regressive phase, the tumor gradually shrinks and changes from bright red to dark gray. Therefore, we should actively intervene early to accelerate the regression of hemangioma, reduce the complications and eliminate the scars on the child's face, which is important for the child's subsequent growth and personal needs.

In the past, surgery was generally used to remove hemangioma, but it is easy to recur because it bleeds a lot during the surgery and is not easy to remove cleanly. Nowadays, surgical resection is no longer the first choice for hemangioma treatment due to the widespread use of sclerotherapy, herbal medicine and radiotracer. Currently, β-blockers are gradually being used in the treatment of infantile hemangiomas(3). However, the mechanism of action, indications and safety of β-blockers as the first-line therapeutic agents for replacing hormones in the treatment of hemangioma have been the focus of attention of basic and clinical workers engaged in hemangioma research. The mechanism of treatment is still unclear, and the concentration and strength of the effect on IH need to be further investigated.

Because IHs occur in infants and children at a younger age, drug selection should be more careful. Although propranolol has become the first choice for the treatment of IHs because of its ability to accelerate the regression of hemangiomas, there are adverse drug reactions such as elevated blood potassium. The safety and efficacy of external treatment of IH with timolol have been recognized. The tumor tissue of proliferating IH consists of a variety of cells, and only hemangioma stem cells (HemSCs)

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form a tumor model in vivo that approximates the natural course of IH. In vitro experiments have now shown that(4) HemSCs can be induced to differentiate into the major components of IHs at various stages of development: endothelial cells, pericytes and adipocytes. Our study was designed to investigate the efficacy of timolol in the treatment of hemangioma and its effect on the proliferation and apoptosis of hemangioma stem cells.

Materials and Methods

General Information

Sixty children with IHs, 32 males and 28 females, aged 3-7 months, mean age (5.14±1.56) months, duration of disease 6-12 d, mean duration (9.21±2.16) d, hemangioma area 2-8 cm2, mean area (5.16±2.48) cm2 were selected and divided into two groups according to the lottery method. In the observation group, there were 30 cases, 16 males and 14 females; age ranged from 4 to 7 months, mean age (5.52 ± 1.26) months; duration of disease ranged from 6 to 11 d, mean duration (8.25 ± 2.14) d; hemangioma area ranged from 2 to 7 cm2, mean area (4.5± 1.81) cm2. In the control group, there were 30 cases, 16 males and 14 females; age ranged from 3 to 7 months, mean age (5.46±1.41) months; disease duration 6-12 d, mean disease duration (8.35±2.26) d; hemangioma area 2-8 cm2, mean area (4.48±1.76) cm2. This study was approved by the ethics committee.

Observation indicators

Efficacy judgment

The four-level classification criteria proposed by Achauer et al.Grade I: no significant change in the tumor (tumor shrinkage ≤25%); Grade II: tumor shrinkage and lighter color (tumor shrinkage 26%~50%); Grade III: tumor shrinkage and lighter color (tumor shrinkage 51%~75%); Grade IV: tumor mostly disappeared (tumor shrinkage >75%). The effective rate was calculated by grade II and above, and grade V was a clinical cure; ultrasound examination: ultrasound scan was performed in the quiet state of the child before and after 6 months of treatment. A Philips EPIQ5 ultrasound instrument with 3D Doppler angiography, 2DL12-5 probe and 3DVL13-5 volumetric probe were used to scan the lesion in 2D to observe the size, location and blood flow; after that, the conventional conditions of small organs were selected, the range was set to 700MHz, the frame frequency was 42%, and the angle was 30°. The 3D gray-scale images and 3D-CPU images were saved. The images were analyzed by QLAB Advanced Ultrasound Quantification Software Release 10.5 software, and the 3D tumor images were reconstructed, and the software automatically calculated the 3D data of the lesion, i.e. volume (V), vascular index (VI), flow index (FI) and vascular flow index (VFI).

HemSCs index

The apoptosis and value-added of HemSCs were determined by the observation and analysis of before and after data.

Incidence of adverse reactions

Regular follow-ups were conducted once a month by telephone and outpatient review, and follow-ups were conducted until six months after treatment, and the incidence of adverse reactions, such as hypertension, eczema and diarrhea, were counted in the children to calculate the incidence of adverse reactions.

Statistical methods

All statistical data were processed by SPSS160 statistical software. The measurement data were first tested for normal distribution, and data conforming to normal distribution or normally distributed after conversion were expressed as mean ± standard deviation. Because FINSHOMA-IR indicators were skewed, natural logarithm conversion was performed, and their natural logarithms obeyed normal distribution after conversion and were treated according to the law of normal distribution. Paired t-test was used to compare the indexes before and after treatment within the group, and two independent samples t-test was used to compare between two groups; the χ^2 test was used for counting data; the Ridit test was used to compare the efficacy of grade data. $p<0.05$ was considered a statistically significant difference.

Results

Comparison of the four-level scores of children in the two groups

Combining the four-level scores and ultrasound results, the number of effective treatment cases in the observation group was 28, which was significantly higher than that in the control group, $P<0.05$, see Tables 1-3, and Figures 1

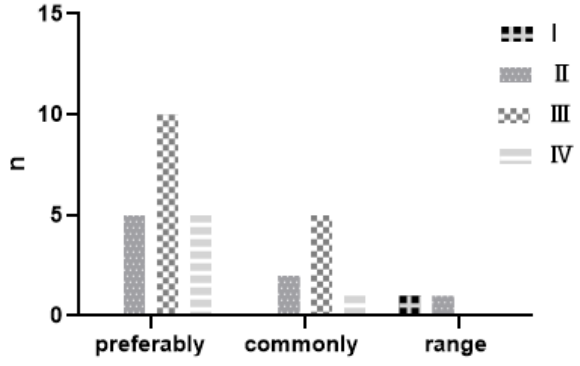

Figure 1. The efficacy results of the observation group. Grade I: no significant change in the tumor (tumor shrinkage ≤25%); Grade II: tumor shrinkage and lighter color (tumor shrinkage 26%~50%); Grade III: tumor shrinkage and lighter color (tumor shrinkage 51%~75%); Grade IV: tumor mostly disappeared (tumor shrinkage >75%).

Table 1. Results of the efficacy of the observation group (n(%)).

Ultrasound results	Four levels of scoring				
	I	II	III	IV	Total
Better	0	5	10	5	20(66.67)
General	0	2	5	1	8(26.67)
Poor	1	1	0	0	26.67)

Table 2. Efficacy results of the control group (n(%)).

	I	II	III	IV	Total
Better	0	2	6	1	9(30)
General	0	7	3	1	11(36.67)
Poor	8	1	1	0	10(33.33)

Table 3. Comparison of treatment effects between the two groups of children (n(%)).

Group	n	Effective	Invalid
Observation group	30	28(93.33)	2(6.67)
Control group	30	20(66.67)	10(33.33)
X2	-	6.667	
P	-	0.010	

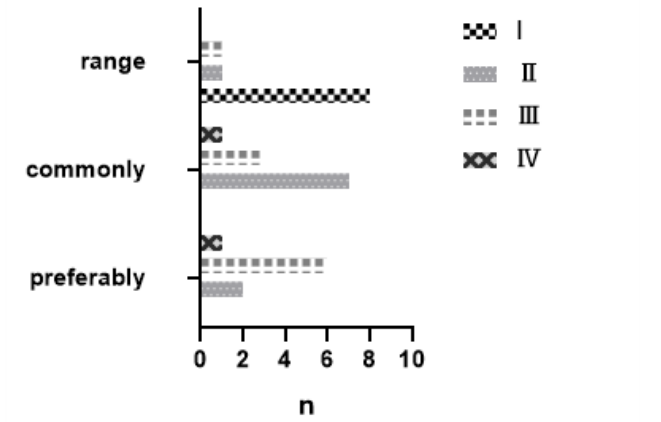

Figure 2. Efficacy results of the control group. Grade I: no significant change in the tumor (tumor shrinkage ≤25%); Grade II: tumor shrinkage and lighter color (tumor shrinkage 26%~50%); Grade III: tumor shrinkage and lighter color (tumor shrinkage 51%~75%); Grade IV: tumor mostly disappeared (tumor shrinkage >75%).

and 2.

Comparison of the incidence of adverse reactions

The total number of adverse reactions in the observation group was 2 cases, with an incidence rate of 6.67%, which was significantly lower than that of the control group, $P<0.05$, see Table 3 and Figure 3.

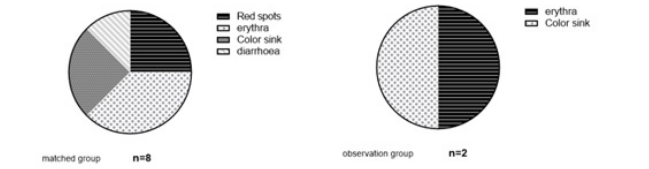

Figure 3. Comparison of the occurrence of adverse reactions in the two groups of children.

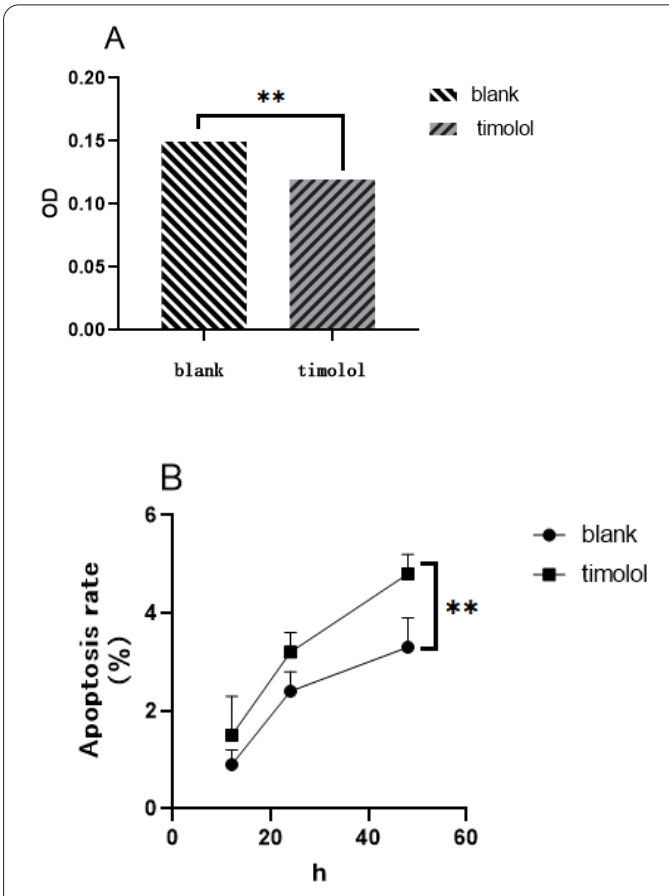

Figure 2. Value-added apoptosis of hemangioma stem cells. A: Results of proliferation assay of hemangioma stem cells. B: Results of apoptosis assay of hemangioma stem cells.

Observation of the value-added apoptosis of hemangioma stem cells

The proliferation level of hemangioma stem cells was significantly inhibited under the intervention conditions of timolol (Figure 4A), and the apoptosis rate of hemangioma stem cells increased significantly ($P<0.05$) with the increase of culture time, in which the apoptosis rate of the timolol group was significantly higher than that of the blank control group at the same time point ($P<0.05$), with the most prominent difference at 48h, $P<0.001$ (Figure 4B).

Discussion

IH is a clinically distinctive, benign endothelial cell tumor in children that can occur in different parts of the body, with 60% of them located in the head and neck affecting the patient's appearance. Depending on the depth of the lesion, there are superficial (located in the papillary dermis), deep (located in the reticular dermis or subcutaneous tissue), and mixed (with both superficial and deep hemangioma features) types. Most infantile hemangiomas can subside on their own after birth, and the natural course of the disease is characterized by distinct stages, with rapid proli-

Table 3. Comparison of the incidence of adverse reactions (cases).

Group	Erythema, flaking	Rash	Color Sink	Diarrhea	Total incidence (%)
Control	2	3	2	1	8 (26.67)
Treatment	0	1	1	0	2 (6.67)
χ^2	-				4.320
P	-				0.037

feration at the beginning, followed by slow regression, and usually complete remission at the end. A very small number of hemangiomas grow in certain important locations of the child's body, and with their rapid growth, they may cause serious body dysfunction and even endanger life (6-7). So active treatment strategies must be adopted in a timely manner. The common treatments for superficial hemangiomas are laser, and topical medications (imiquimod, hormones). The first three are dedicated to the treatment of superficial lesions, while the latter two have effects on both superficial and deep lesions. Pulsed dye laser treatment for superficial hemangiomas is recommended by many dermatologists and is relatively safe because it destroys hemoglobin through selective photothermal action. However, for periocular hemangioma, it is difficult for children to cooperate with, difficult to treat, expensive, and still has a certain chance of causing blistering, hyperpigmentation, hypopigmentation, skin texture change, ulceration, scarring(8), especially thin skin around the eyes, which is less tolerable and has various adverse effects that are difficult for parents to accept. Other lasers such as emerald laser, and erbium laser CO laser effect are inferior to pulsed dye laser, and complications are significantly increased. Oral propranolol has been recognized by most international and domestic scholars as the first-line drug for the treatment of hemangioma, and no serious complications have been reported. However, propranolol has effects on all systems(9), while topical timolol can significantly reduce the effects of the drug on the whole body and only work on local lesions, which is safer. Timolol is originally an ophthalmic drug. It is a non-selective and potent beta receptor antagonist, with blocking effects on both beta1 and beta2 receptors,and 8 times stronger than propranolol, with no endogenous sympathomimetic activity, no direct cardiac inhibition, and a significant hypotensive effect(10-11) on the eye. Clinically, it is mainly used for the treatment of hypertension, angina pectoris tachycardia and glaucoma, which is mainly characterized by rapid onset of action, few adverse effects, good tolerability, and no effect on pupil size, response to light and visual acuity. Therefore, when using timolol for the treatment of hemangioma, there is no need to worry about vision damage caused by the drug entering the eye. As an ophthalmic drug, timolol has very little irritation to local tissues, and in theory, it does not cause significant local skin effects. Moreover, the efficacy of the topical drug is basically limited to the local area, and the absorption and reaction to the whole body are extremely small, so timolol is very safe as a topical drug, and the experience of 30 children in the observation group of this study also proved this, and no local and systemic adverse reactions were seen.

The present study showed that the number of treated people in the observation group was 28, and the effective rate was 93.33%, which was significantly higher than the control group. It indicates that topical timolol can effectively treat facial hemangioma. Combined with the effect of timolol on hemangioma stem cells, the reasons can be analyzed as follows: timolol, as a β-blocker, can reduce vascular blood flow by blocking receptors causing vascular smooth muscle contraction in the early stage of IH thereby. In addition, endothelial type NO synthase (eNOS) plays an important role in vascular remodeling and angiogenesis associated with some arteriovenous malformations. Some scholars found that the expression of eNOS protein decreased after the use of β-blockers in IH patients. It is speculated that β-blockers can reduce the release of vasodilator NO after inhibiting the expression of eNOS thus causing vasoconstriction of the tumor(12-13), in clinical The improvement of IH patients can be observed, with softening of the hemangioma body, reduction of its size and lightening of its color. At the same time, two pro-angiogenic factors, basic fibroblast growth factor (BFGF) and vascular endothelial growth factor (VEGF), are involved in the growth process of IH. It has been found that β-blockers inhibit cell production and angiogenesis by down-regulating the RAF-mitogen-activated protein kinase pathway(14) and reducing the expression of VEGF and bFGF genes, thereby inhibiting the growth of hemangiomas. It has also been found that β-blockers can inhibit the cell growth process and thus induce the regression of hemangioma cells by downregulating the PI3K/AkV/eNOS/VEGF pathway(15), which decreases the expression levels of NO and VEGF. Matrix metalloproteinase-9 (MMP-9) plays a key role in the process of endothelial cell migration and tubular formation Inhibition of MMP-9 secretion can achieve a reduction in the in vitro invasion and angiogenesis of human microvascular endothelial cells. Li Liqin et al. found(16) that propranolol treatment reduced urinary MMP-9 excretion in IH patients, suggesting that its molecular pathway may represent a molecular target for this drug. The renin-angiotensin system has been shown to play an important role in the development of IH. High systemic renin levels may be responsible for the rapid proliferation of infantile hemangiomas, but it is also possible that the local renin-angiotensin system, which may explain the observed response of IH to topical application of timolol, by suppressing local renin levels and eventually reducing angiotensin II levels, leads to proliferative IH reduced proliferation of vascular endothelial cells. In addition, the main role of apoptosis-inducing B-blockers in the regressive phase of IH is to accelerate tumor regression by inducing apoptosis. It has been suggested that(17-18), propranolol may induce apoptosis by downregulating the expression of CD147 in human umbilical vein endothelial cells. It has also been suggested that the possible mechanism is to induce apoptosis in IH cells by reducing VEGF while increasing angiopoietin 2 (Ang2) levels9. In addition, some experiments(19-20) have also found that propranolol promotes the rate of apoptosis in tumor cells by downregulating the expression of the Bcl-2 gene and NF-kB to achieve tumor regression. In the present study, we found that in the treatment of IH with 0.5% timolol

maleate solution, timolol maleate could selectively induce apoptosis of endothelial cells and accelerate tumor regression. Zhang Yunfei and Wang Wanjun(21-22) et al. found that the inhibitory effect of timolol on the proliferation activity of hemangioma endothelial cells (HemEC) was gradually increased in a dose-dependent manner, which confirmed that timolol significantly inhibited the proliferation of HemEC and promoted the apoptosis of HemEC.

In conclusion, timolol maleate can be used as the first choice of topical drug for superficial IH without systemic adverse effects, with better safety than oral propranolol, and it is a safer treatment method because of its easy administration, good tolerability, and high local concentration in the tumor. The present study was not fully analyzed due to the small number of cases and is not generalizable; it is advisable to increase the number of cases for an in-depth study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not Applicable.

Availability of data and materials
The simulation experimental data used to support the findings of this study are available from the corresponding author upon request.

Competing of Interest
The authors declared that they have no conflicts of interest regarding the publication of this paper.

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Authors' contributions
HJ Z wrote the main manuscript. H L prepared the data collection. WJ L analyse and interpret of results. All authors reviewed the results and approved the final version of the manuscript.

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