Clinical efficacy of pingyangmycin-assisted hemangioma surgery at peculiar sites in children and effects on expression of ER, VEGF and bF-GF proteins

Dingan Wu1, Yanqing Yang1, Guoliang Pi2, Yong Zhang3

1Tongren Hospital of Wuhan University (Wuhan Third Hospital), Wuhan 430060, Hubei, China; 2Hubei Cancer Hospital, Wuhan 430079, Hubei, China; 3Wuhan Children’s Hospital, Tongji Medical College, Huazhong University of Science & Technology, Wuhan 430015, Hubei, China

Received November 7, 2018; Accepted May 7, 2019; Epub August 15, 2019; Published August 30, 2019

Abstract: Hemangioma is a common vascular disease in infants and young children and some tumor masses locate at peculiar sites that damage the function of organs and may even affect development. However, treatment of infantile hemangioma at a peculiar site is not uniform. This study evaluated the clinical efficiency of the treatment ‘pingyangmycin-assisted resection’. A total of 129 children with hemangioma at a peculiar site was selected, and were divided into three groups randomly. Simplex surgical removal of tumor (group A), pingyangmycin local injection (group B), and pingyangmycin local injection combined with surgery procedure (group C) were performed. Short-term clinical efficacy, one-year recurrence rate, complication during treatment & adverse drug reaction rate, and protein expression of ER, VEGF and bF-GF in tumor tissue was taken into consideration to evaluate the clinical efficiency. After three courses of treatment, the clinical total effective rate among three groups was significantly different, group C was higher than group B and the cure rate of group C were higher than group A and B (P<0.05). After one-year follow-up visit, the recurrence rate of group C was lower than group A and B (P>0.05). The complication during treatment and adverse drug reaction rate among the three groups was not statistically different and all patients were able to tolerate adverse drug reactions, and the complications disappeared after symptom treatment. The positive expression rate of ER, VEGF, and bF-GF protein in post-treatment tumor tissues of patients in group C was significantly lower than group B and C (P<0.05). Treatment, with local injection of pingyangmycin combined with surgical resection of hemangioma at a peculiar site, can effectively reduce the positive expression of ER, VEGF and bF-GF protein in tumor tissues. Clinical efficacy was solid with lower recurrence rate, and children could tolerate postoperative complications and adverse drug reactions. The results suggest that this treatment approach has high clinical value.

Keywords: Infantile hemangioma, pingyangmycin local injection, estrogen receptor, vascular endothelial factor, basic fibroblast factor

Introduction

Hemangioma is a common vascular disease in infants and young children, which is accompanied by abnormal proliferation of vascular endothelial cells and benign tumors in blood vessels [1]. As infants grow, approximately 70% of infantile hemangioma could completely resolve at seven years old. However, the clinical data show that about 25%-69% of the recovered children who didn’t receive treatment had residual skin and subcutaneous tissue degeneration in the tumorous area, such as scars, atrophy and dermatolysis. Therefore, the appearance of skin is affected as well as their future life [2]. The children with moderate to high-risk infantile hemangioma are recommended for surgical resection to achieve a complete cure. However, due to some peculiar site tumor masses (hemangioma in maxillofacial region), the effect of surgical resection is not consistent, which makes constant clinical efficacy of hemangioma at a peculiar site an important subject [3]. Recent studies showed that the pathogenesis and development of hemangioma are related to the positive expression of vascular endothelial growth factor (VEGF), estrogen (ER), and basic fibroblast growth fac-
tor (b-F-GF) [4-6]. In this study, pingyangmycin-assisted surgical resection was used to treat hemangioma at a peculiar site. The clinical efficacy and expression of ER, VEGF and b-F-GF protein in tumor tissue were observed to provide preferential treatment of hemangioma at a peculiar site.

Materials and methods

Inclusion and exclusion criteria

A total of 129 children with hemangioma at peculiar site (hemangioma developed in the maxillofacial region), treated in the hospital from November 2014 to February 2016, were selected. They were numbered according to the date of treatment and divided into group A, B and C randomly, with 43 patients in each group. There was no significant difference in patients’ age, gender, the location of hemangioma, pathological stage, and the positive expression rates of ER, VEGF, and b-F-GF protein in pre-treatment tumor (Table 1).

Inclusion criteria: (1) age ≤2 years old; (2) in line with the diagnostic criteria for infantile hemangiomas of “Classification of Hemangioma and Vascular Malformation” made by International Classification of Hemangioma and Vascular Deformity Society in 2014 [7]; (3) all patients’ tumor was in proliferative phase; (4) the state of illness was classified as medium/high risk according to the “Risk Level and Division Basis of Hemangiomas” [8]; (5) met the indications for surgical treatment; (6) tolerate with Pingyangmycin; (7) the family members were aware of the advantages and disadvantages of participating in the study, informed consent was signed, and were willing to cooperate during research.

Exclusion criteria: (1) those who did not meet the above inclusion criteria; (2) parents did not sign the informed consent. (3) Patients had experienced bronchial asthma, mycoplasma pneumonia and glomerulonephritis in recent 6 months.

Experiment design and treatment

All patients were treated with B-mode ultrasound, angiography, etc. to determine the extent of the lesion, pathological type, and tumor invasion. Surgery plan was confirmed for those needed surgical treatment. Patients in group A received surgical resection, group B received pingyangmycin (produced by Jilin Jidong Pharmaceutical Group Yanji Co., Ltd., National Pharmaceutical Standard H20123357, specification: 8 mg (based on pingyangmycin hydrochloride)) local injection (8 mg of pingyangmycin was dissolved in 2 ml physiological saline, after shaking and dissolving, it was injected into the hemangioma tissue at a dose of 1 ml of pingyangmycin per 1 cm² tumor area. When the color of the tumor turned white, the injection was stopped. For tumor areas larger than 1 cm², 2-3 injections were applied at different locations, one injection every two weeks in one course of treatment, and three courses for continuous treatment were performed. Group C was treated with surgical resection combined with pingyangmycin injection (pingyangmycin was injected into the residual tumor after resection, the subsequent injection was the same as group B). During the treatment, the color of the tumor, adverse drug reaction, and the postoperative complications were observed.

Observation objects

Short-term clinical efficacy, one-year recurrence rate, complication during treatment & adverse drug reaction rate were compared among the three groups. ER, VEGF, b-F-GF protein expression in tumor tissues were detected (patients’ tumor tissue was sampled at pre-
Statistical analysis

Statistical analysis was performed using SPSS 18.0 (SPSS, Inc., Chicago). The enumeration data were analyzed by the \( \chi^2 \) test, the rank data were analyzed by rank sum test, the measurement data were analyzed by t-test, and the multi-group measurement data were analyzed by variance analysis. When the statistical difference of variance analysis was significant, the subsequent pairwise comparison was performed by LSD-t/Games-Howell test, \( P<0.05 \) was defined as the difference was statistically significant.

Results

Clinical efficacy among different treatment groups

After three courses of treatment, the clinical total effective rate among three groups was significantly different, group C was significantly higher than group B. The cure rate of group C were significantly higher than group A and group B (\( P<0.05 \), Table 2). After one year of follow-up visit, the recurrence rate of group C was lower than group A and group B. The recurrence rate of group A was lower than group B. The differences were not statistical different (\( P>0.05 \), Table 3).

During treatment, the adverse drug reaction rate & postoperative complication rate of group A was lower than group C (\( P<0.05 \)). All patients could tolerate adverse drug reactions, and the complications disappeared after symptomatic treatment (Table 4).

Comparison of ER, VEGF, and bFGF protein expression pre and post-treatment among the three groups

The positive expression rate of ER, VEGF, and bFGF protein in post-treatment tumor tissues of patients in group C was significantly lower.

---

### Table 2. Clinical efficacy among the three groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Cure (%)</th>
<th>Almost cure (%)</th>
<th>Improved (%)</th>
<th>Invalid (%)</th>
<th>Clinical total effective rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (n=43)</td>
<td>25 (58.14)</td>
<td>10 (23.26)</td>
<td>3 (6.98)</td>
<td>5 (11.63)</td>
<td>38 (88.37)</td>
</tr>
<tr>
<td>B (n=43)</td>
<td>20 (46.51)</td>
<td>11 (25.58)</td>
<td>4 (9.30)</td>
<td>8 (18.60)</td>
<td>35 (81.40)</td>
</tr>
<tr>
<td>C (n=43)</td>
<td>32 (74.42)</td>
<td>7 (16.28)</td>
<td>3 (6.98)</td>
<td>1 (2.33)</td>
<td>42 (97.67)</td>
</tr>
</tbody>
</table>

Fisher exact test \( x^2 \) value: 6.139, \( P \) value: 0.041

### Table 3. One-year recurrence rate among the three groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of cases</th>
<th>Recurrent cases</th>
<th>Recurrence rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>43</td>
<td>3</td>
<td>6.98</td>
</tr>
<tr>
<td>B</td>
<td>43</td>
<td>5</td>
<td>11.63</td>
</tr>
<tr>
<td>C</td>
<td>43</td>
<td>1</td>
<td>2.33</td>
</tr>
</tbody>
</table>

Fisher exact test \( x^2 \) value: 2.754, \( P \) value: 0.295

---

ER, VEGF and bFGF protein expression in tumor tissues

All patients’ tumors were sampled by fine needle aspiration, pre-treatment, one month and one year after treatment. Samples were dehydrated and paraffin embedded. 4 µm tissue slides were made followed by immunohistochemistry staining using the SP method. Protocol and results evaluation were modified according to Shin [10, 11].
Treatment of infantile hemangiomas

Table 4. Comparison of adverse drug reactions and complications during treatment and follow-up among the three groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of cases</th>
<th>Postoperative infection cases (%)</th>
<th>Gastrointestinal reaction cases (%)</th>
<th>Mucosal ulcer cases (%)</th>
<th>Complication after operation &amp; adverse drug reaction cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>43</td>
<td>2 (4.66)</td>
<td>-</td>
<td>-</td>
<td>2 (4.66)</td>
</tr>
<tr>
<td>B</td>
<td>43</td>
<td>-</td>
<td>2 (4.66)</td>
<td>3 (6.98)</td>
<td>5 (11.63)</td>
</tr>
<tr>
<td>C</td>
<td>43</td>
<td>1 (2.33)</td>
<td>2 (4.66)</td>
<td>4 (9.31)</td>
<td>7 (16.28)*</td>
</tr>
</tbody>
</table>

Fisher exact test $x^2$ value: 4.972  
$P$ value: 0.087  
Note: *Comparison with group A, $P<0.05$.

Table 5. ER, VEGF and bF-GF protein expression pre and post-treatment among the three groups ($\bar{x} \pm s, n=43$)

<table>
<thead>
<tr>
<th>Group</th>
<th>Pretreatment (%)</th>
<th>1 month after treatment (%)</th>
<th>1 year after treatment (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ER</td>
<td>VEGF</td>
<td>bF-GF</td>
</tr>
<tr>
<td>A</td>
<td>73.7±13.0</td>
<td>69.5±18.4</td>
<td>66.6±11.5</td>
</tr>
<tr>
<td>B</td>
<td>73.9±9.7</td>
<td>71.0±17.8</td>
<td>67.8±10.6</td>
</tr>
<tr>
<td>C</td>
<td>72.3±11.8</td>
<td>69.6±14.0</td>
<td>69.4±11.5</td>
</tr>
</tbody>
</table>

F value: 0.248  
$P$ value: 0.781

Note: *In group comparison with pretreatment data ($P<0.05$); #post-treatment data in comparison with group A ($P<0.05$).

than group A and B ($P<0.05$). Group A was lower than group B except for ER ($P<0.05$) (Figure S1). The intragroup comparison of positive expression rate of ER, VEGF, and bF-GF protein at different point-in-time showed a significant difference ($P<0.05$) (Figures S2 and S3). The intergroup comparison at the same treatment stage also revealed different expression rate, with only a few not being statistically significant ($P>0.05$) (Table 5).

Discussion

Infantile hemangioma is formed by vascular tissue hyperplasia during the embryonic period, characterized by abnormal proliferation of vascular endothelial cells, and the site of occurrence is non-specific but mainly in the skin and soft tissue [12]. The skin or soft tissue of children with hemangioma shows congestive, scab-like or telangiectasia spots in the early stage. Hemangiomas are involuted naturally, and physicians normally prefer to observe with limited intervention [13]. However, the tumor mass will grow rapidly with the surface bulges on the skin, if children with infantile hemangiomas enter the early proliferative phase at six months after birth. Most children’s tumor cell ceases to proliferate before the age of one, and a few children continue to proliferate after one year old [14].

The local injection of pingyangmycin combined with surgical resection in treating hemangioma shows a significant advantage in this study. There are certain clear indications for operative therapy, and the selection of the treatment modality depends on the type, location, and size of the lesions as well as the treatment cost and techniques available. Pingyangmycin is similar to bleomycin A5 and is made in China. It has been isolated from the many compounds of bleomycin produced by Streptomyces pingyangensis. Molecular analysis showed that pingyangmycin belongs to a subbranch of bleomycin A5. Zheng et al. found that pingyangmycin prevents DNA repair by inhibiting DNA ligase [15]. In addition, pingyangmycin has a specific sclerosing effect on vascular endothelium.

The present studies have confirmed that a variety of endothelial cytokines, angiogenic factors, growth factors, vascular endothelial cell receptor family and bone marrow markers are highly expressed among the proliferative hemangioma tissues, while the accelerated endothelial cell apoptosis, high expression of mast cell and metalloproteinase inhibitors are observed in regressive hemangioma tissues [16, 17]. These results reveal that the occurrence and development of hemangiomas may be the
result of vascular endothelial cell abnormal proliferation caused by abnormalities in endothelial cell transformation.

It is well accepted that the infantile hemangioma needs immediate treatment, but without any conclusion to summarize the positive and side effects among different treatment programs. Simplex drug treatment such as bleomycin, pingyangmycin and some anti-tumor drugs are effective, but it is hard to hold the appropriate dosage, especially for infants with poor body tolerance. Clinical research shows that over-dosage of the drugs mentioned above may lead to developmental delay or disorder in the injection sites. Some patients will still have the apparent appearance or functional problems after simplex drug treatment [13, 18, 19]. Although, surgical procedure advanced in improving appearance, removing lesions, cosmetic reconstruction and improving dysfunction [20], it is difficult to remove the tumor completely for the children who has peculiar tumor site and large tumor size. Therefore, this study used pingyangmycin-assisted surgical resection to treat children with hemangioma at peculiar site. The results show that the pingyangmycin-assisted surgical resection had a preferable prognosis and the lower recurrence rate, compared with the simplex pingyangmycin local injection and simplex surgery. Compared with expression of ER, VEGF, and bF-GF protein in the pre and post-treatment tumor tissues, the expression rate was significantly lower, indicating that pingyangmycin-assisted surgery method could kill tumor cells more thoroughly, and it is speculated that the long-term recurrence rate would be lower.

In total, local injection of pingyangmycin combined with surgical resection in treating hemangioma at peculiar site patients can effectively reduce the positive expression of ER, VEGF and bF-GF protein in tumor tissues. Clinical efficacy is solid with low recurrence rate, and the children can tolerate postoperative complications and adverse drug reactions that could be considered with high clinical value.

Disclosure of conflict of interest

None.

Address correspondence to: Yong Zhang, Wuhan Children’s Hospital, Tongji Medical College, Hua- zhong University of Science & Technology, Wuhan 430015, Hubei, China. E-mail: Publetscientific@126.com

References

Treatment of infantile hemangiomas


Figure S1. Expression of ER protein in tumor tissues from different groups.
Figure S2. Expression of VGEF protein in tumor tissues from different groups.
Figure S3. Expression of bF-G protein in tumor tissues from different groups.