Propranolol as an Alternative Treatment Option for Pediatric Lymphatic Malformation

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Lymphatic malformation (LM), which was previously termed lymphangioma, is a rare congenital malformation of the lymphatic system and its treatment is still challenging. Propranolol (beta blocker) has been recently developed as a first-line treatment of infantile hemangioma. Our study aimed to assess the effect of propranolol on pediatric LM and the relationship between its effectiveness and vascular endothelial growth factor (VEGF) family members (VEGF-A, C and D). Six Japanese patients with LM (age range: 10 months-19 years old; 2 macrocystic, 2 microcystic and 2 combined type) were enrolled. Oral propranolol was administered at 2 mg/kg/day. The efficacy of propranolol for LM was evaluated by the rate of volume change as calculated from MRI imaging and by symptomatic improvement. In all patients, there were no significant side effects. Patients 3 and 5 were classified as objective responders with tumor volume reduction of 30.6% and 22.9%, respectively, at 24 weeks. Patient 1 showed 8% tumor volume reduction and patient 6 showed symptomatic improvement, hence, both were classified as minimal responders. The other two patients were classified as non-responders. Plasma VEGF-A, C, and D levels were significantly higher in the LM group than in the controls (all \( P < 0.01 \) by Mann-Whitney test). VEGF-A and D levels at 24 weeks were significantly lower than those at pre-treatment (\( P = 0.031, 0.047 \) by Wilcoxon matched pairs test). Though further trials with this treatment must be carried out, we propose that propranolol may be an alternative therapy option for intractable LM.

Keywords: beta blocker; lymphangioma; lymphatic malformation; propranolol; vascular endothelial growth factor


Lymphatic malformation (LM) is a rare congenital malformation of the lymphatic system, which usually occurs in children before the age of two years (Wiegand et al. 2008). LM was previously termed lymphangioma. Surgical excision and sclerotherapy have been used for LM. However, local recurrence is common and the complication rate is high (Marler and Mulliken 2005). Therefore, the treatment of LM remains challenging.

Propranolol is a non-selective beta blocker that is used for the treatment of a variety of cardiovascular diseases. Since the report of Léauté-Labrèze et al. (2008), propranolol has been widely used for the first-line treatment of infantile hemangioma (IH). We reported successful propranolol treatment of intractable diffuse lymphangiomatosis with thoracic involvement (Ozeki et al. 2011). Propranolol is thought to cause down-regulation of the Raf mitogen-activated protein kinase signaling pathway, with reduced expression of vascular endothelial growth factor (VEGF) (Storch and Hoeger 2010). Because plasma VEGF levels in our diffuse lymphangiomatosis patient were high before treatment and were reduced after successful treatment, we hypothesized that propranolol inhibits lymphangiogenesis and reduces LM growth by inhibition of VEGF. Multiple lesions with increased VEGF staining have been reported in LM specimens histologically (Sidle et al. 2005). Therefore, we considered that propranolol could have a beneficial effect on LM as in diffuse lymphangiomatosis.

The VEGF family is involved in the development and growth of the vascular endothelial system. Originally VEGF-A, the founding member of the VEGF family, was simply termed VEGF. VEGF-A is a potent growth factor for blood vessel endothelial cells. VEGF-C and VEGF-D have recently been recognized as playing a role as lymphatic system regulators (Alitalo and Carmeliet 2002).
We report a clinical trial of propranolol administered to patients with LM and the relationship between plasma levels of VEGF family members (VEGF-A, C and D) and the effectiveness of propranolol treatment.

Methods

Patients

This study protocol was approved by the Ethical Committee of the Graduate School of Medicine, Gifu University. Consent to review patient records and for treatment of propranolol (off-label indication) was received from patients or parents whose children participated in this study in accordance with our institutional ethical standards. Inclusion criteria were as follows: aged from 1 month to 20 years; a stable clinical condition before the study; no history of asthma; reactive airway disease; impaired renal dysfunction; heart defects; arrhythmia; or central nervous system disorders.

Study protocol

The study design is detailed in Fig. 1. Before the start of treatment, the protocol included a clinical examination, echocardiography, electrocardiogram, recording of baseline heart rate, blood pressure, and clinical photographs. Patients were admitted for 5 days of observation at the initiation of treatment. On the first day, oral propranolol was administered at 0.5 mg/kg/day, divided into 3 doses, it was increased to 1 mg/kg/day on the second day, if tolerated well, and further increased to 2 mg/kg/day from day 4, which is well below the dose given for IH. Vital signs and blood sugar levels were monitored 1 hour after administration of each dose of medication, and continuous electrocardiogram monitoring was performed during the patients’ sleep. Follow-up visits were performed every 4 weeks, including clinical examination, measurement of vital signs, and clinical photographs. Apart from medication with propranolol, no alternative or adjuvant therapies were performed. The response to propranolol treatment was assessed clinically and radiologically. Digital photographs were taken by the same primary physicians who produced standardized images, using the same views and settings as in the baseline image. Magnetic resonance imaging (MRI) was performed at pre-treatment, and at 12, 24 and 48 weeks after the initiation of treatment. Tumor volumes were calculated by using MRI in both coronal and sagittal views. The primary outcome was the rate of tumor reduction and symptomatic improvement at 24 weeks. Assessments of response were classified as a good response if there was a reduction in size greater than 50%. Patients showing a degree of improvement greater than 10% and less than 50% were rated as an objective response. Patients showing minimal improvement less than 10% or transient improvement of symptoms were classified as having a minimal response. Finally, patients showing no improvement were classified as no response.

Laboratory tests for plasma VEGF levels during treatment

Peripheral blood samples were obtained at the time of pretreatment, and at 4, 8, 12, and 24 weeks after the initiation of treatment. All blood samples were centrifuged at 1,000 × g for 30 minutes. Plasma was separated, aliquoted, and stored at −80°C until analysis was performed. Plasma VEGF levels were determined using a commercially available monoclonal antibody-based enzyme-linked immunosassay kit designed to measure each type of VEGF according to the manufacturer’s instructions (Quantikine; R&D Systems, Minneapolis, MN, USA). The sensitivities of VEGF-A, C, and D were 5.0 pg/ml, 4.0 pg/ml, and 4.7 pg/ml, respectively. Optical density was measured at 450 nm using an automated microplate reader. We also measured VEGF levels from samples from 30 control children ranging from 2 months to 17 years old (mean ± SE: 3.9 ± 0.7 years old) for references.

Statistical analyses

Significance of differences in the VEGF levels between the LM group and control group before treatment was tested using the Mann-Whitney test. Significance of differences in VEGF levels between pre-treatment and 24 weeks after treatment was tested using Wilcoxon matched pairs test. A P value of less than 0.05 was considered statistically significant. All analyses were performed using GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA).

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Assessment

Treatment

Propranolol (2 mg/kg/day)

Fig. 1. Study protocol.

The LM patients underwent propranolol therapy, and its safety and efficacy were determined during a routine schedule.
Results

Patient characteristics are shown in Table 1. All patients were evaluated and treated at our institution between 2010 and 2012. A total of 6 patients (2 boys and 4 girls; age range: 10 months-19 years old; mean ± SE: 6.7 ± 3.6 years old) were enrolled. LM was identified at birth in all patients. We classified LM into 3 subtypes as follows: cysts larger than 2 cm in diameter were classified as “macrocystic”; those smaller than 2 cm in diameter were “microcystic”; and mixed lesions were “combined”. Treatment modalities used before propranolol therapy consisted of sclerotherapy (OK-432 injection) and an operation. All patients included in the study were able to complete the treatment program, and there were no dropouts.

In six patients, there were no good responders. Two patients (patients 3 and 5) showed an objective response and LM was decreased in the initial stage of treatment. The clinical course of objective responders was as follows.

Patient 3, a 10-month-old boy, presented with a large, soft-tissue mass in the left lower leg since birth. At the time of admission, there was limited motion of the left ankle joint because of subcutaneous edema (Fig. 2A). Gadolinium-enhanced MRI showed a mixture of enhanced and non-enhanced lesions in the subcutaneous tissue of the lower thigh and toe (Fig. 2B). Surgical resection or sclerotherapy was considered to be difficult because of the risk of recurrence and complications. The patient was enrolled in our study with the consent of his parents. Several days after initiation of treatment, the skin turgor gradually decreased and skin creases appeared. After 4 weeks, the leg swelling was diminished and the joint stiffness had improved. The percentage of the calculated volume of the total lower leg was reduced (Fig. 2C). The gadolinium-enhanced lesion was predominantly diminished. The volume of the dorsum of the foot, which was not enhanced by gadolinium, increased in parallel with the growth of bone and muscle.

Patient 5, a 1-year-old boy, presented with a combined (macrocystic and microcystic) LM of the left neck (unilateral, infrahyoid, and suprahypoid) since birth. At 20 days of age, partial resection was performed to reduce dyspnea, and the histological diagnosis of LM was made. The tumor grew continuously and he received a local injection of OK-432 for the macrocystic LM at 1 month of age. However, the sclerotherapy resulted in airway obstruction and airway intubation was necessary for 1 month. At 1 year of age, because surgical resection of the whole lesion appeared to be difficult, he was referred to our hospital to receive propranolol treatment (Fig. 2D). Several days after initiation of treatment, the pericystic skin gradually softened. Similar to patient 3, the MR gadolinium-enhanced lesion showed a reduction in volume, and the paratracheal lesion disappeared (Fig. 2E). The percentages of calculated volumes of the total LM and gadolinium-enhanced lesion were reduced at 24 weeks (Fig. 2F).

Two patients (patients 1 and 6) showed minimal reduction of less than 10% or transient improvement of symptoms. Patient 1, a 19-year-old woman with macrocystic LM of the left neck and mediastinum, showed a minimal reduction of 8% at 4 weeks. Patient 6, a 17-year-old woman, with combined LM of the right neck and maxillo-
facial area, had pain and bleeding from microcystic lesions of the oral mucosa. Several days after treatment, the bleeding stopped and the pain disappeared. No change was observed in the size of LM. Two patients (patients 2 and 4) showed no response.

In all patients, there was no significant side effect during treatment. The two teenagers (patients 1 and 6) had transient lightheadedness and a mild headache. We evaluated hemodynamic variables during admission and on follow-up visits. Transient bradycardia was observed in 1 patient. This episode occurred at night during sleep and was self-limiting. Overall, the adverse events were all mild and transient, and there was no drop-out of patients because of adverse events.

**VEGFs at pretreatment and during treatment**

Prior to therapy, plasma VEGF-A, C and D levels were significantly higher in the LM group than those in the control group (all \( P < 0.01 \)). VEGF-A and D levels at 24 weeks after treatment were significantly lower than those at pre-treatment (\( P = 0.031, 0.047 \), respectively). Although VEGF-C levels did not significantly change between pre-treatment and 24 weeks after treatment, VEGF-C levels in objective responders (patients 3 and 5) appeared to be decreased after treatment compared with those in minimal and non-responders (patients 1, 2, 4 and 6). We could not evaluate the statistical analysis because the number of patients was small.

**Discussion**

We conducted a clinical trial of propranolol therapy for pediatric LM. Among 6 patients, patients 3 and 5 were objective responders who showed obvious symptomatic improvement and had a greater than 10% and less than 50% shrinkage in their tumor volume. The gadolinium-enhanced lesion as shown by MRI was predominantly diminished in these patients. Two patients had some palliation of symptoms, although tumor volume reduction was less than 10%. Hemorrhage from oral mucosal LM was quickly stopped in patient 6. LM patients had higher plasma VEGF levels than those in pediatric controls, and they were decreased by propranolol treatment. These patterns were more evident in
objective responders. Propranolol might be more effective in patients with gadolinium-enhanced lesions as shown by MRI and high plasma VEGF levels, as discussed below.

Currently, propranolol is widely used and is recommended as the first-line treatment for IH in some sites, especially the airway (Peridis et al. 2011). Treatment of intractable LM is still challenging, especially in patients of cervical LM, such as in patient 5, who was treated by partial resection and sclerotherapy, but whose mixed-type LM had not regressed after 1 year (Fig. 2D). Moreover, he had to be intubated because of airway obstruction caused by inflammation after sclerotherapy. However, propranolol treatment had a favorable effect without an adverse reaction (Fig. 2D). Patient 3 had microcytic LM in his lower leg and had functional problems for walking. This type of LM invades the surrounding structures, so the outcome of surgical resection is generally unsatisfactory. Propranolol showed an objective response and functional improvement (Fig. 2A). Propranolol therapy was apparently effective in intractable LM in the patients who had a reduction in tumor volume.

Patient 6 had clinical improvement for oral bleeding from LM lesion after initiation of propranolol, although no obvious reduction in LM volume was observed. Similar observations have been reported in patients with lingual LM lesions (Leboulanger et al. 2011). Beta blockers inhibit the vasodilation mediated by adrenaline via beta-adrenergic receptors, and this leads to vasoconstriction. Vasoconstriction is reported as an early effect of propranolol on IH (Storch and Hoeger 2010). Propranolol treatment may affect hemorrhagic lesions and improve the quality of life in those who have mucosal bleeding from LM lesions.

The effect of propranolol on reduction of tumor volume varied among patients, and even within LM lesions in patients. We performed an objective assessment using MRI-based volumetric measurements of LM. The results of our study demonstrated that gadolinium-enhanced lesions decreased after treatment in patients 3 and 5. LM in the other 4 patients had no apparent gadolinium-enhanced lesions, and had only a minimum reduction in volume. In LM, gadolinium-enhanced lesions consist of hypervascular or mixed vascular lesions. Therefore, propranolol may have a positive effect on these lesions through blood flow because propranolol treatment reduces lesion volume and vessel density in patients of IH (Bingham et al. 2012). Propranolol might be more effective for LMs with gadolinium-enhanced lesions than for those without such lesions.

Propranolol leads to a reduced expression in VEGF and, therefore, causes an inhibition of angiogenesis (Storch and Hoeger 2010). This effect is one of the important mechanisms of regression of IH. Serum levels of VEGF are elevated in infants during the proliferative phase of IH.
Conversely, the expression of VEGF is significantly reduced during the involution phase, as well as in completely regressed LMs. Propranolol treatment in a patient with intractable diffuse lymphangiomatosis resulted in a reduction in plasma VEGF levels in parallel with clinical improvement. Reduced expression of VEGFs by propranolol causes down-regulation of the extracellular signal-related kinase / mitogen-activated protein kinase cascade, which is important for angiogenesis. Recent studies suggest that VEGFs can act as lymphangiogenic factors (Ferrara 2004). VEGF-C is strongly expressed in patients of microcystic LM compared with patients with other types of LM, suggesting that these patients possess proliferative activity (Itakura et al. 2009). In our study, plasma VEGF levels in LM patients were significantly higher than those in pediatric controls. After treatment, plasma VEGF levels were significantly decreased, especially in objective responders. Plasma VEGF may be a reliable marker of activity of LM and a good indicator of response to therapy.

Our study limitations included a heterogeneous patient population and a small number of patients. Larger trials may confirm these results and provide more detailed information. Medical treatments, including interferon and systemic corticosteroids, have been attempted for patients with extensive inoperable LM, with varying degrees of success. Although these drugs generally have serious side effects, propranolol has been used to safely treat cardiac conditions in children for over 40 years and it is well tolerated with few adverse effects. In conclusion, we propose that propranolol is an alternative treatment for challenging LMs.

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Conflict of Interest

The authors declare no conflict of interest.

References