Original Paper

Imatinib Ameliorated Retinal Neovascularization by Suppressing PDGFR- α and PDGFR- β

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Key Words

Retinal neovascularization • Imatinib • PDGFR- α • PDGFR- β

Abstract

Background/Aims: Platelet-derived growth factors (PDGFs) have emerged as pivotal in pathological angiogenesis, which is a hallmark of various tumors and retinal diseases. Here we evaluated the anti-angiogenic effect of imatinib, an inhibitor of PDGF receptors α and β (PDGFR- α and - β), in retinal neovascularization using an oxygen-induced retinopathy (OIR) model. **Methods:** The OIR model was established and given imatinib or vehicle treatments daily from P12 to P16. At the peak of angiogenesis at P17, the neovascularization area was quantified on retinal whole-mounts with isolectin B4 staining. Immunofluorescence staining and western blots were used to determine the effect of imatinib on different vascular cells and the pathway molecules involved. **Results:** Imatinib effectively suppressed pathological angiogenesis in OIR mice and reduced the number of all three types of vascular cells, including endothelial cells, pericytes, and smooth muscle cells. Moreover, the expression and activation of PDGFR- α and - β were inhibited by imatinib. The imatinib-treated OIR mice presented with reduced expression of other potent pro-angiogenic factors such as VEGF and FGF2. No obvious retinal or systemic side effects were observed in the imatinib treatment group. **Conclusions:** Imatinib appears to be safe and effective in suppressing retinal neovascularization. Targeting PDGFs/PDGFRs may also be important for anti-angiogenic treatment and offer a viable alternative treatment for retinal angiogenic diseases.

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Introduction

Retinal neovascularization (NV) is the main pathological feature of many vision-threatening diseases including retinopathy of prematurity, diabetic retinopathy, age-related macular degeneration, and retinal vein occlusion [1, 2]. The development of NV is tightly

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regulated by endogenous angiogenesis-related proteins [3, 4]. Among them, vascular endothelial growth factor (VEGF) has emerged as a potent inducer that targets the vascular endothelial cells (ECs) as the main effector [5]. Since 2004, anti-VEGF agents have been widely used for treating retinal NV diseases in clinical practice. However, increasing evidence suggests that anti-VEGF therapy has limited efficacy in nonresponsive and drug-resistant patients. Angiogenesis may become VEGF-independent at a more advanced stage and thus responds poorly to VEGF inhibitors [6]. In addition, pathological angiogenesis comprises abnormal proliferation and migration of ECs, pericytes (PCs), and smooth muscle cells (SMCs). The latter two are not typical cellular targets of VEGF and show limited response to anti-VEGF therapy [6-9]. Therefore, novel anti-angiogenesis strategies that target PCs and SMCs are strongly desired.

Platelet-derived growth factors (PDGFs) are considered to play an important role in vascular homeostasis through the activation of the tyrosine kinase receptors PDGFR- α , and PDGFR- β . Both receptors could be expressed on ECs, PCs, and SMCs, and PDGFs have broad biological functions in vasculature under various pathological conditions [10, 11]. In the eye, PDGFs were reported to be pivotal pro-angiogenic factors involved in choroidal, retinal, and corneal NV via activation of PDGFRs in both VEGF-dependent and -independent manners [12, 13]. Notably, the PDGFs/PDGFRs axis exerts a distinct effect compared with VEGF/VEGFRs due to their considerably broad cellular targets. The angiogenic effect of PDGFs has been shown to be as potent as that of VEGF in many angiogenesis models [13, 14]. In addition, some studies have reported that patients who respond poorly to anti-VEGF therapy have elevated levels of PDGFs [15], indicating the alternative pro-angiogenic effect of the PDGFs/PDGFRs axis. These findings suggest that inhibition of PDGFs/PDGFRs might be a promising therapy for suppressing angiogenesis, either alone or in combination with anti-VEGF therapy.

Imatinib, a specific tyrosine kinase inhibitor mainly targeting Bcr-Abl, c-Kit, and PDGFR- α and - β , has been successfully used in treating chronic myeloid leukemia and gastrointestinal stroma tumors (GIST) through the inhibition of Bcr-Abl and c-Kit [16]. In addition, imatinib, a potent inhibitor of PDGFR- α and - β , was used to inhibit angiogenesis in tumor models such as vestibular schwannoma tumor and craniopharyngioma [17, 18]. However, the anti-angiogenic effect of imatinib in the retina remains largely unknown. Given the important role of the PDGFs/PDGFRs axis in retinal angiogenesis, we speculated that imatinib might be effective in reducing retinal NV via the inhibition of PDGFRs. In this study, we aimed to determine the anti-angiogenic effect of imatinib in retinal NV and to elucidate the cellular and molecular mechanism involved using oxygen-induced retinopathy (OIR), a classical retinal NV mouse model.

Materials and Methods

Mice and OIR model

C57BL/6J mice were purchased from Guangzhou University of Chinese Medicine and maintained in a specifically pathogen-free facility at the Animal Laboratories of the Zhongshan Ophthalmic Center. This study adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. All the experimental procedures were approved by the Institutional Animal Care and Use Committee of Zhongshan Ophthalmic Center, Sun Yat-Sen University. The OIR model was established as previously described [19]. In brief, C57BL/6J mouse pups with their nursing mothers were placed in an Oxy Cyclor system (BioSpherix, Inc., Parish, NY, USA) with $75 \pm 2\%$ oxygen from postnatal day 7 (P7) to P12. At P12, pups were returned to room air. The mice were sacrificed at P17, and their eyes were enucleated for further analysis.

Imatinib treatment and evaluation of side effects

Imatinib (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 0.9% saline solution to a final volume of 100 μ l and injected intraperitoneally into the mice once a day from P12 to P16 [20-22]. Different doses of imatinib (25 mg/kg/d, 50 mg/kg/d, and 100 mg/kg/d) were used to evaluate its effect on retinal

angiogenesis. An equal volume of 0.9% saline solution was used as a vehicle control. The retinas were collected at P17, and sections were prepared for hematoxylin & eosin (H&E) staining and TUNEL assays to evaluate retinal toxicity. To evaluate the systemic side effects of imatinib, the body weights of mice were measured daily from P12 to P17, and the spleen, liver, and kidney were weighed after mice were sacrificed.

Isolectin B4 staining on retinal whole-mounts

Eyes were fixed in 4% paraformaldehyde (PFA) for 1 h at room temperature. Retinas were carefully removed and incubated in 1% bovine serum albumin (BSA) containing 0.3% Triton X-100 in phosphate-buffered saline (PBS) at 4°C overnight. FITC-conjugated isolectin B4 (IB4; 1:50; Shanghai Invitrogen Biotechnology Co., Ltd., Shanghai, China) was used to visualize the vasculature of the retina. After being washed extensively, retinas were mounted on slides. Images were captured using fluorescent microscopy (DM4000, Leica, Germany). The area of neovascular tufts was quantified using Photoshop (CC 2015; Adobe Systems, San Diego, CA, USA).

Immunofluorescence staining and TUNEL assay on retinal cryosections

Eyes were fixed in 4% PFA for 1 h at room temperature. After being washed with PBS, eyes were dehydrated in 30% (w/v) sucrose for 12 h and then cryoprotected in Tissue-Tek (Sakura Finetek USA Inc., Torrance, CA, USA) at -20°C. Eight-micrometer vertical retinal cryosections along the nasotemporal axis were prepared. After being incubated in 1% BSA, retinal cryosections were immunostained with primary antibodies at 4°C overnight. The primary antibodies included anti-cluster of differentiation 31 (CD31) antibody (1:100; Becton Dickinson, Franklin Lakes, NJ, USA); anti-smooth muscle actin (SMA) antibody (1:100; Dako, Glostrup, Denmark); anti-neural/glial antigen 2 (NG2) antibody (1:200; Millipore, Billerica, MA, USA); anti-VEGF, anti-fibroblast growth factor 2 (FGF2), and anti-glial fibrillary acidic protein (GFAP) antibodies (1:200; Abcam, Cambridge, MA, USA); and anti-PDGFR- α and anti-PDGFR- β antibodies (1:50; Santa Cruz Biotechnology Inc., Dallas, TX, USA). After being rinsed with PBS, retinal cryosections were incubated with secondary antibodies for 1 h at room temperature. The secondary antibodies included Alexa Fluor 647-conjugated donkey anti-rat IgG, Alexa Fluor 555-conjugated donkey anti-mouse IgG, and Alexa Fluor 488-conjugated donkey anti-rabbit IgG (1:800; Abcam). TUNEL staining (In Situ Cell Death Detection Kit, Fluorescein; Roche, Indianapolis, IN, USA) was performed according to the manufacturer's instructions. Retinal cryosections were stained with DAPI for 5 min at room temperature and visualized using confocal microscopy (Carl Zeiss LSM710, Oberkochen, Germany).

H&E staining

After fixation with 4% formalin, eyes were embedded in paraffin and cut into 3- μ m-thick vertical slices. Sections were washed in deionized water for 5 min and treated with hematoxylin buffer for 10 min at room temperature. The sections were rinsed in deionized water and then dipped in 1% Eosin solution for 15 s. After being rehydrated in an alcohol gradient, slices were mounted. Histological analyses of retinal tissues were observed under a microscope (Leica DM4000, Wetzlar, Germany) to detect neovascular nuclei anterior to the internal limiting membrane (ILM).

Western blot analysis

Retinal protein samples were harvested and homogenized in lysis buffer (RIPA, Biocolors, Shanghai, China) containing protease and phosphatase inhibitor mini tablets (Thermo Fisher Scientific, No.88668; Waltham, MA, USA). The primary antibodies included anti-PDGFR- α , anti-phosphorylated PDGFR- α (p-PDGFR- α), anti-PDGFR- β , anti-p-PDGFR- β , and anti-hypoxia-inducible factor 1 α (HIF-1 α) antibodies (1:200; Santa Cruz Biotechnology, Dallas, TX, USA) as well as anti-extracellular signal-regulated protein kinases 1 and 2 (Erk1/2) and anti-p-Erk1/2 antibodies (1:1000; Cell Signaling Technology, Beverly, MA, USA). β -actin (Abcam) was used as a loading control. After being washed with TBST (Tris-buffered saline and Tween 20), the membranes were incubated with a horseradish peroxidase-conjugated secondary antibody at a concentration of 1:2000 for 1 h at room temperature. The signals were developed with Super Signal West Dura extended duration substrate (Thermo Fisher Scientific, Waltham, MA, USA), and the images were captured by an image station.

Statistical analysis

All experiments were repeated at least three times. Student's *t*-tests were applied using Prism 5 software (GraphPad Software Inc., San Diego, CA, USA). The data are reported as the mean $\pm$ standard deviation (SD). A *p*-value of less than 0.05 was accepted as statistically significant.

Results

Imatinib could ameliorate retinal NV in the OIR model

Imatinib is a potent inhibitor of PDGFR- α and - β , for which signaling plays an important role in retinal angiogenesis. We first treated the OIR mice with different doses of imatinib (25 mg/kg/d, 50 mg/kg/d, or 100 mg/kg/d) to evaluate its potential effect on retinal NV. IB4 staining was performed on retinal whole-mounts to visualize angiogenesis. As shown in Fig. 1A, there was no significant reduction of NV areas under the imatinib treatment at 25 mg/kg/d in comparison with the vehicle treatment, indicating a limited anti-angiogenic effect of imatinib at this dose. Notably, OIR mice under the imatinib 50 mg/kg/d or 100 mg/kg/d treatment presented with marked decreases in NV areas, indicating the potent anti-angiogenic effect of imatinib at these doses. Then the 50 mg/kg/d dose was selected for the following experiments. In addition, endothelial cell nuclei beyond the ILM were counted in retinal sections to evaluate the neovascular tufts protruding into the vitreous. As shown in Fig. 1B, the pre-retinal endothelial cell nuclei were significantly reduced in the imatinib-treated group (50 mg/kg/d) compared with the vehicle control group. These data revealed that imatinib can prevent pathological angiogenesis in the OIR model.

Imatinib suppressed proliferation of vascular ECs, PCs, and SMCs

Since PDGFR- α and - β could be expressed on different kinds of vascular cells, we next investigated the target cells of imatinib in retinal NV. Retinas were stained with CD31, SMA, and NG2, the markers for ECs, SMCs, and PCs, respectively. Imatinib treatment led to decreased numbers of CD31⁺ and SMA⁺ cells compared with the vehicle-treated group (Fig. 2A and C). Consistently, the numbers of CD31⁺ and NG2⁺ cells were also reduced by imatinib administration (Fig. 2B and C), suggesting that imatinib inhibited the proliferation of these three types of vascular cells. In addition, there were no differences in the ratios of CD31/NG2 and CD31/SMA expression between the two groups, indicating that imatinib simultaneously targets all the vascular cells and suppresses their proliferation (Fig. 2D).

Fig. 1. Imatinib ameliorated retinal neovascularization in OIR model. (A) Mice were intraperitoneally injected with different doses of imatinib (25 mg/kg/d, I-25; 50 mg/kg/d, I-50, and 100 mg/kg/d, I-100) or vehicle (0.9% saline solution) daily from P12 to P16. At P17, eyes were enucleated for retinal whole-mounts and IB4 staining. The NV area was quantified and presented as the percentage to total area of retina. Imatinib treatment at 50 mg/kg/d or 100 mg/kg/d suppressed markedly retinal angiogenesis with similar reduction of NV areas, while imatinib treatment at 25 mg/kg/d showed no significant reduction of NV areas. Scale bars: 1000 μ m. *n*=6. (B) Representative images of H&E-stained retinal sections showed that the cell nuclei above ILM layer (arrows) in imatinib-treated mice (50 mg/kg/d) were much less than control. Scale bars: 50 μ m. *n*=6 eyes and 6 sections/eye. *, *P*<0.05; **, *P*<0.01. n.s.: no significance.

Fig. 2. Imatinib suppressed the amount of vascular endothelial cells (ECs), pericytes (PCs) and smooth muscle cells (SMCs). (A) Retinal sections were co-labeled with SMCs marker SMA (red) and ECs marker CD31 (green). Representative images showed decreased SMA⁺ and CD31⁺ cells after imatinib treatment (50 mg/kg/d). The boxed regions are shown at higher magnification on the above images. Scale bar: 100 μ m. (B) Quantification of CD31⁺, NG2⁺, SMA⁺ cells per 630X microscope field. n=6 eyes and 6 sections/eye. (C) Retinal sections from mice treated with vehicle or imatinib were co-labeled with PCs marker NG2 (red) and CD31 (green). Representative images showed reduction of NG2⁺ and CD31⁺ cells in the imatinib-treated group. (D) Statistical analysis of the ratios of CD31/NG2 and CD31/SMA expression in the vehicle- or imatinib-treated retinas. n=6 eyes and 6 sections/eye. **, P<0.01; ***, P<0.001. n.s.: no significance.

Fig. 3. Imatinib suppressed the expression and activation of PDGFR- α and PDGFR- β and inhibited HIF-1 α and Erk1/2 signalings in OIR retina. (A, B) Immunofluorescence assay on retinal sections was performed to detect the expression of PDGFR- α (A) and PDGFR- β (B). Representative images showed increased expression of PDGFR- α and - β , which were co-labeled with CD31⁺ ECs, NG2⁺ PCs and SMA⁺ SMCs, in OIR retina compared to controls. After imatinib treatment, the expression of PDGFR- α and - β was reduced. Scale bars: 100 μ m. (C, D) Western blotting was performed to detect the expression of PDGFR- α , phosphorylated(p)-PDGFR- α (C), PDGFR- β , and p-PDGFR- β (D) in vehicle- and imatinib-treated OIR retina. Note that Imatinib treatment led to decreased expression of PDGFR- α and - β and their activated forms. (E) The expression of HIF-1 α was significantly increased in OIR retina compared with control, however, this effect was abolished by imatinib treatment. (F) The expression and phosphorylation of Erk1/2 was evaluated by western blot. Compared with non-OIR control, the OIR retina showed increased phosphorylation of Erk1/2. After imatinib treatment, the expression of p-Erk1/2 was markedly decreased. β -actin was used as reference.

Imatinib suppressed the expression and activation of PDGFR- α and PDGFR- β and inhibited the HIF-1 α and Erk1/2 pathways during OIR

We further investigated the expression of PDGFRs using immunofluorescence staining and found that both PDGFR- α and - β were highly up-regulated in OIR retinas compared with the non-OIR controls. Notably, the increased PDGFRs were mainly co-located with CD31⁺, SMA⁺, and NG2⁺ vascular cells. After imatinib treatment, the expression levels of PDGFR- α and - β were significantly reduced, as well as the number of vascular cells (Fig. 3A and B).

Western blots showed that imatinib treatment could reduce the protein level of PDGFR- α and - β as well as phosphorylated (p)-PDGFR- α and p-PDGFR- β , indicating that imatinib could markedly inhibit the expression and activation of PDGFR- α and - β in OIR retinas (Fig. 3C and D). In addition, activation of PDGFR- α and - β was reported to upregulate HIF-1 α , the key component of the HIF pathway involved in hypoxia-induced angiogenesis [23]. We detected HIF-1 α expression by western blot and found imatinib treatment abrogated the elevation of HIF-1 α expression in OIR retinas (Fig. 3E). Furthermore, Erk1/2 signaling, which plays an important role in promoting the survival, proliferation, and migration of vascular cells, was detected, and the western blot results revealed reduced phosphorylation of Erk1/2 in the imatinib-treated group compared with the vehicle group (Fig. 3F), indicating that imatinib may suppress retinal NV in part through inhibiting the phosphorylation of Erk1/2.

Imatinib reduced the expression of potent angiogenesis factors in angiogenic retinas

To determine whether imatinib treatment affects other potent angiogenesis factors, we detected the expression of FGF2 and VEGF, two classical pro-angiogenic factors in OIR retinas

Fig. 4. Imatinib reduced the expression of other potent angiogenesis factors in angiogenic retina. (A) Representative images of retinal sections co-stained with FGF2 and EC marker CD31 (Left) or SMC marker SMA (Right). Note that after imatinib treatment, ECs and SMCs expressed less angiogenic FGF2. Scale bars: 100 μ m. (B) Representative images of retinal sections co-labeled with VEGF and CD31 (Left) or müller/astrocytes marker GFAP (Right). Expression of VEGF from ECs and müller/astrocytes was reduced after imatinib treatment.

Fig. 5. Imatinib treatment had no obvious retinal and systemic side-effect. Imatinib (50 mg/kg/d) was administered intraperitoneally in OIR mice daily from P12 to P16. (A) H&E staining showed retinal structure and thickness in the vehicle- and imatinib-treated mice. No significant difference in total thickness of INL (inner nuclear layer) and ONL (outer nuclear layer) was observed between the two groups. Notably, the structure of imatinib-treated retina was well-organized. Scale bars: 50 μ m. (B) TUNEL staining on retinal sections showed similar amounts of apoptotic cells during OIR in two groups. Scale bars: 100 μ m. (C) The body weight of littermates was measured daily from P12 to P17 and no significant difference between vehicle- and imatinib-treated mice was observed. (D) At P17, the weight of critical organs including liver, spleen, and kidney were also evaluated and no significant differences were found. n=6. n.s.: no significance.

treated with imatinib. In the vehicle group, strong immunostaining of FGF2 was observed in the inner retina, which was mainly co-stained with CD31⁺ and SMA⁺ cells (Fig. 4A). Notably, imatinib treatment downregulated FGF2 expression on ECs and SMCs. In addition, as shown in Fig. 4B, VEGF was abundantly expressed in the CD31⁺ ECs and GFAP⁺ cells (a marker for Müller cells or astrocytes) in the vehicle-treated OIR retinas, while imatinib treatment could reduce VEGF expression on these cells.

No obvious retinal or systemic side effects were observed in the imatinib-treated OIR mice

To determine the safety of imatinib treatment for OIR mice, H&E staining and TUNEL assays were first performed to evaluate potential retinal toxicity. As shown in Fig. 5A, H&E staining of retinal sections revealed the well-organized structure of imatinib-treated retinas and the nearly equal thickness of the inner and outer nuclear layers, in which most neural cells are located, in both the vehicle- and imatinib-treated groups. In addition, no difference in TUNEL positive apoptotic retinal cells was observed between the two groups, indicating no potential retinal toxicity of imatinib (Fig. 5B). Furthermore, whole body weight and the weight of vital organs, such as the liver, spleen, and kidney, were measured. The mean weight of imatinib-treated mice was 6.23 ± 0.11 g compared to 6.06 ± 0.33 g for the vehicle-treated mice at P17, and no significant difference was observed at each day from P12 to P17 (Fig. 5C). In addition, the weight of the liver, spleen, and kidney also showed no significant difference between the imatinib and vehicle groups (Fig. 5D). These data suggest that imatinib treatment did not induce obvious retinal or systemic side effects in the OIR mice.

Discussion

In the present study, we demonstrated for the first time that imatinib could effectively ameliorate retinal NV by inhibiting PDGFR- α and - β in OIR mice. Importantly, our finding revealed that imatinib treatment reduced the number of several types of vascular cells and inhibited other potent pro-angiogenic factors, highlighting the important role of the PDGFs/PDGFRs axis in retinal pathological angiogenesis. Targeting the PDGFs/PDGFRs axis with imatinib may provide a new strategy for anti-angiogenesis therapy, extending the scope for treating angiogenic diseases.

PDGFs/PDGFRs exert several complex effects including stimulating angiogenesis, remodeling the nascent vascular networks, and modulating vascular permeability in vascular systems under physiological and pathological conditions [24]. The angiogenic effect induced by PDGFs/PDGFRs has been demonstrated to be both VEGF-dependent and VEGF-independent [13, 25], with the latter mechanism being concentrated. Indeed, PDGFs/PDGFRs have been shown to play a pivotal role in promoting tumor angiogenesis in a VEGF-independent manner in certain types of tumors that are resistant to anti-VEGF therapy. Additionally, anti-VEGF therapy could increase the expression of PDGFs, suggesting the activation of PDGFs/PDGFRs as a compensatory pathway in the absence of VEGF and the PDGFs/PDGFRs axis as a promising target for the treatment of angiogenic diseases [15, 26, 27]. Imatinib was reported to inhibit the expression and activation of PDGFRs *in vitro* and *in vivo* by targeting the ATP-binding site of tyrosine kinases of PDGFRs and inhibiting their biological effect [28, 29]. In the present study, we found that imatinib could effectively ameliorate retinal angiogenesis by suppressing the expression and phosphorylation of PDGFRs in the OIR model, suggesting that imatinib may be a novel anti-angiogenesis drug used either alone or in combination with anti-VEGF therapy.

Angiogenesis develops a neovascular network, which requires not only ECs but also the cooperation of PCs and SMCs [30, 31]. The newly formed vessels were initiated by ECs and matured by PCs and SMCs. This cooperation orchestrates the angiogenesis process [32]. Therefore, targeting single vascular cell types, such as ECs, might achieve poor anti-angiogenic effects in certain situations [6]. In the present study, we found imatinib treatment simultaneously decreased the numbers of CD31⁺ ECs, SMA⁺ SMCs, and NG2⁺ PCs in the OIR

model, suggesting a broad inhibitive effect of imatinib on vascular cell types, which was different from that of anti-VEGF agents. In addition, we found imatinib could inhibit the phosphorylation of Erk1/2, which is involved in the survival, proliferation, and migration of vascular cells and is recognized as part of an important signaling pathway closely involved in angiogenesis [33], suggesting that the reduction of angiogenesis by imatinib may occur in part through inhibiting Erk1/2 signaling pathways. Oxidative stress plays an important role in retinal angiogenesis [34, 35]. HIF, the pivotal mediator of cellular responses to oxygen stress, was decreased by imatinib treatment [35]. This result suggests that imatinib might also suppress angiogenesis by ameliorating oxidative stress in retinas. Further study is needed to elucidate the molecular mechanism involved in the action of imatinib on oxidative stress.

Interestingly, we found imatinib could also reduce the expression levels of VEGF and FGF2 in the OIR retina. This observation is in line with previous reports that PDGF-CC could up-regulate VEGF production [15]. VEGF was also reported to act as an inducer of indirect action on other angiogenic factors, such as FGF2 and PDGF [36, 37]. FGF2 is another considerably important factor that could regulate VEGF expression in ECs and may also play an integral role in resistance to anti-VEGF therapy [38]. These angiogenic factors may cooperate with each other to orchestrate functional neovascular formation and maturation. The present study revealed that inhibition of PDGFRs by imatinib reduced the expression of VEGF and FGF2 and effectively suppressed the process of angiogenesis, further suggesting complex interactions between these pro-angiogenic factors. In addition, it was reported that PDGF induced VEGF and FGF2 expression by activating the Erk signaling pathway in multiple angiogenesis models [39, 40]. We also found imatinib suppressed the phosphorylation of Erk in the OIR retina, so it is reasonable to speculate that inhibition of the ERK pathway is involved in the reduced expression of VEGF and FGF2 by imatinib.

Imatinib is able to selectively inhibit the tyrosine kinase activity of several molecules such as Bcr-Abl and c-Kit, has been successfully used in treating malignant diseases, and is generally well tolerated [41]. Bcr-Abl is a well-known fusion gene that is expressed exclusively in chronic myeloid leukemia cells, and c-Kit was identified as an oncogenic tyrosine kinase involved in various hematological malignancies and solid tumors [16]. In addition, imatinib could also inhibit the tyrosine kinase activity and signaling of PDGFRs. Based on the potent inhibiting effect of PDGFRs, imatinib showed some efficiency in treating non-malignant diseases, such as systemic sclerosis, pulmonary arterial hypertension, hyperlipidemia, and atherosclerosis [42, 43]. In the present study, we found both PDGFR- α and - β are widely expressed in SMCs, PCs, and ECs in retinas, and imatinib prevented retinal angiogenesis in OIR through PDGFR- α and - β . The most common side effects of imatinib treatment include cutaneous reactions, edema, diarrhea, and nausea [44]. In the present study, we used imatinib at a dose of 50 mg/kg/d, which was reported to block PDGFR signaling efficiently in mice with low or no toxicity [20, 21]. We found imatinib treatment did not induce obvious retinal or systemic side effects in this OIR model.

In summary, this study revealed that imatinib ameliorated retinal angiogenesis by inhibiting PDGFR- α and - β in the OIR model. Furthermore, imatinib could simultaneously act on three types of vascular cells, namely, ECs, SMCs, and PCs, which might enable it to act with a more extensive anti-angiogenic effect than current treatments. Targeting PDGFs/PDGFRs with imatinib may thus be important for anti-angiogenic treatment and offer a viable alternative treatment for angiogenic diseases.

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Disclosure Statement

The authors declare to have no competing financial interests.

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