

Review



Toward the Clinical Application of Therapeutic Angiogenesis Against Pediatric Ischemic Retinopathy

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ABSTRACT

Therapeutic angiogenesis refers to strategies of inducing angiogenesis to treat diseases involving ischemic conditions. Historically, most attempts and achievements have been related to coronary and peripheral artery diseases. In this review, we propose the clinical application of therapeutic angiogenesis for the treatment of pediatric ischemic retinopathy, including retinopathy of prematurity, familial exudative retinopathy, and *NDP*-related retinopathy. These diseases are all characterized by the reduction of physiological angiogenesis and the following induction of pathological angiogenesis. Therapeutic angiogenesis, which supplements insufficient physiological angiogenesis, may be a therapeutic approach for ischemic conditions. Various molecules and modalities can be utilized to apply therapeutic angiogenesis for the treatment of ischemic retinopathy, as in coronary and peripheral artery diseases. Experiences with cardiovascular diseases provide a useful reference for the further clinical application of therapeutic angiogenesis in pediatric ischemic retinopathy. Recombinant proteins and gene therapy are powerful tools to deliver angiogenic factors to retinal tissues directly. Furthermore, endothelial progenitor or bone marrow-derived cells can be injected into the vitreous cavity of the eye for therapeutic angiogenesis. Intraocular injections are highly promising for the delivery of therapeutics for therapeutic angiogenesis. We expect that therapeutic angiogenesis will be a breakthrough in the treatment of pediatric ischemic retinopathy.

Keywords: Retinal diseases; Ischemia; Physiologic neovascularization; Angiogenesis modulating agents; Therapeutics

INTRODUCTION

Therapeutic angiogenesis was proposed as a therapeutic approach targeting clinical problems due to the local rarefaction of blood vessels, insufficient neovascularization, or both.^{1,2} After the first suggestion in 1993, ongoing active attempts have been made to apply therapeutic angiogenesis to the treatment of cardiovascular diseases.³⁻⁶ Therapeutic angiogenesis employs various angiogenic factors, including vascular endothelial growth factors (VEGFs), fibroblast growth factors (FGFs), and hepatocyte growth factor (HGF), to induce neovascularization in ischemic tissues. Although preclinical studies of protein,

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Conflict of Interest

The authors have no conflicts of interest to declare.

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gene, and cell therapies were promising, therapeutic angiogenesis is still not a mainstay treatment option for coronary and peripheral artery diseases after the modest success of clinical trials. In this review, we instead suggest that pediatric ischemic retinopathy might be a promising target of therapeutic angiogenesis. Based on the lessons from clinical studies on cardiovascular diseases and recent preclinical studies on ischemic retinopathy, the clinical application of therapeutic angiogenesis to treat patients with pediatric ischemic retinopathy should be investigated through a mechanism-based approach. The eye is an easily accessible organ for the local delivery of therapeutic materials in protein, gene, and cell therapies. This characteristic may help apply modalities of therapeutic angiogenesis in a more feasible way for the treatment of ischemic retinopathy.

PEDIATRIC ISCHEMIC RETINOPATHY

Pediatric ischemic retinopathy refers to a spectrum of retinal diseases characterized by hypovascularization-related ischemia of the retinal tissue (**Fig. 1**). In patients with retinopathy of prematurity (ROP), familial exudative vitreoretinopathy (FEVR), and *NDP*-related retinopathy, there are areas without retinal vessels (known as avascular retina) in the peripheral retina due to the insufficient development of retinal vasculature. The presence of insufficient retinal vessels results in an ischemic retinal microenvironment.

ROP

ROP is a vision-threatening retinopathy involving abnormal retinal vascular development in premature infants, as its name implies (**Fig. 1A**).⁷ The incidence and severity are related to low birth weight and gestational age.⁸ Because the retinal vasculature is not complete in premature infants, the peripheral retina is without retinal vessels. Hypoxia in the peripheral retina activates hypoxia-mediated signaling pathways, increasing the secretion of angiogenic factors.⁷ This, in turn, induces a fibrovascular reaction in the retinal tissues. The stages of ROP reflect the degree of fibrovascular proliferation along the borders of the avascular and vascularized retina. The severity increases from the demarcation line (stage 1) to ridge with volume (stage 2), extraretinal fibrovascular proliferation (stage 3), and tractional retinal detachment (stage 4, partial; stage 5, total).⁷

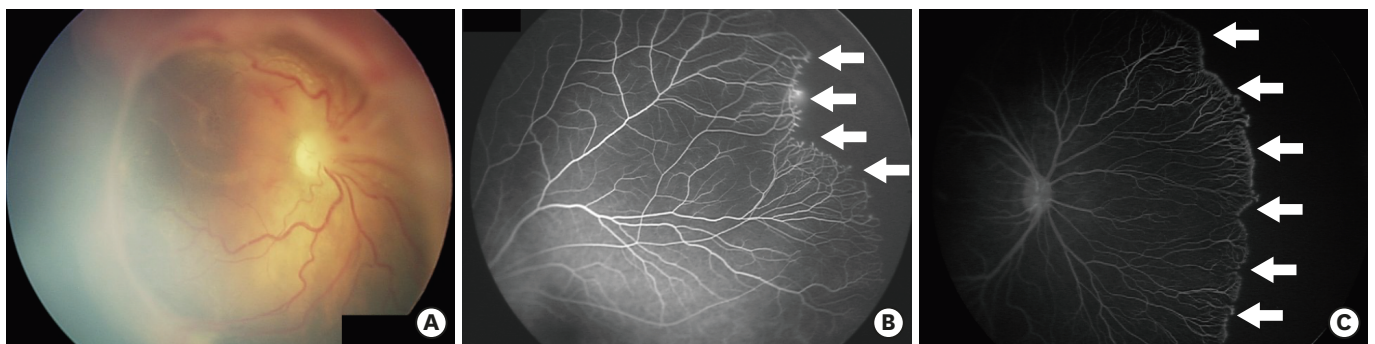


Fig. 1. Representative clinical photographs of patients with pediatric ischemic retinopathy. (A) A fundus photograph of a patient with retinopathy of prematurity. (B) A fluorescein angiography photograph of a patient with familial exudative vitreoretinopathy. (C) A fluorescein angiography photograph of a patient with *NDP*-related retinopathy. The margins between vascularized and avascular retinas are indicated with white arrows.

Animal models that mimic the pathogenesis and consequences of ROP have been developed.^{2,9,10} These models are valuable tools to investigate the potential of therapeutic approaches for ROP. The most widely utilized model is an oxygen-induced retinopathy (OIR) model in mice.^{9,10} In this model, neonatal pups are exposed to 75% oxygen for 5 days from postnatal day 7 (P7) to P12. This procedure leads to the regression of central vessels. Then, the mice in normal room air (20% oxygen) experience relative hypoxia (due to the rarefaction of the retinal vessels) in the central retina, which peaks at P14. As in patients with ROP, neovascular tufts develop along the area between the avascular and vascularized retina. Usually, pathological neovascularization peaks at P17. If therapeutic approaches are developed to recover from rarefaction of the retinal vasculature, it will be possible to prevent the following pathological neovascularization.

FEVR

FEVR is another vision-threatening retinopathy involving retinal hypovascularization in infants and children (**Fig. 1B**).¹¹ The principal feature of the disease is an avascular peripheral retina, as in ROP.¹² This leads to pathological retinal neovascularization in the peripheral retina, with or without exudates and further retinal detachment.¹³ It is noteworthy that patients with FEVR possess variants in genes encoding molecules of the norrin/frizzled class receptor-4 pathway, one of the Wnt/ β -catenin signaling pathways, including *NDP*, *FZD4*, *LRP5*, and *TSPAN12*.¹⁴ Norrin (encoded by the *NDP* gene) is a Wnt ligand, frizzled class receptor-4 (coded by the *FZD4* gene) is a receptor, and low-density lipoprotein receptor-related protein 5 (from the *LRP5* gene) and transpanin 12 (from the *TSPAN12* gene) are coreceptors with frizzled class receptor-4.

As in patients with FEVR, *Ndp* or *Fzd4* knockout mice demonstrated insufficient retinal vasculature compared to wild-type mice.^{15,16} Angiogenesis takes place through multiple steps: 1) the activation of endothelial cells by angiogenic factors, 2) the invasion and protrusion of new sprouts, 3) the proliferation of endothelial cells to support sprout elongation, 4) lumen formation to build vessel loops, and 5) the initiation of blood flow, the establishment of a basement membrane, and the recruitment of mural cells to stabilized new vessels.¹⁷ Primary retinal endothelial cells from *Fzd4* knockout mice demonstrated a lower potential to migrate and form tubules in *in vitro* angiogenesis assays.¹⁵ In addition, *Ndp* knockout mice showed less proliferation of endothelial cells in the retinal vasculature *in vivo*. Because insufficient frizzled signaling leads to retinal hypovascularization in mutant mice and possibly in patients with FEVR, therapeutic strategies to restore aberrant frizzled signaling might be of therapeutic potential.

1. *NDP*-related retinopathies

Mutations in the *NDP* gene have been reported in patients with a spectrum of retinal diseases, including FEVR and ROP.¹⁸⁻²³ In this context, *NDP*-related retinopathies have been proposed to include X-linked FEVR, ROP, persistent hyperplastic primary vitreous, Norrie disease, and Coats disease.²⁴ Although the manifestations of *NDP*-related retinopathies vary, incomplete retinal vascular vasculature is a common feature of these conditions (**Fig. 1C**). Other phenotypes include fibrous stalk in persistent hyperplastic primary vitreous, exudates in Coats disease, and fibrovascular membranes in FEVR, ROP, and Norrie disease, which are provoked and exacerbated by ischemia due to incomplete retinal vasculature. *NDP*-related retinopathies are mainly induced by mutations in the *NDP* gene and resultant insufficiency of the norrin protein. In this context, *NDP* gene therapy and norrin protein supplementation may be useful therapeutic approaches.

2. Common pathological mechanisms and current treatment options of pediatric ischemic retinopathy

It is noteworthy that insufficient physiological angiogenesis is a crucial feature of ischemic retinopathy. This leads to hypoxia-mediated pathological neovascularization, which results in bleeding, exudate formation, and fibrovascular proliferation.^{25,26} Nevertheless, current treatment options for ischemic retinopathy only target pathological neovascularization or its complications, such as vitreous hemorrhage and retinal detachment. In patients with pediatric ischemic retinopathy, laser photocoagulation destroys the retinal tissues in the peripheral vascular retina, suppressing the metabolic demand and the secretion of angiogenic and inflammatory factors.^{7,12} In addition, to minimize VEGF-mediated pathological neovascularization, anti-VEGF antibody (bevacizumab; Genentech, South San Francisco, CA, USA) is administered to patients with ischemic retinopathies such as ROP, FEVR, and diabetic retinopathy.²⁷⁻²⁹ Vitreous hemorrhage and retinal detachment are managed by surgery, such as vitrectomy and encircling. Unfortunately, there are no clinically proven approaches directly targeting retinal hypovascularization for the treatment of ischemic retinopathy.

THERAPEUTIC ANGIOGENESIS

Therapeutic angiogenesis is a direct therapeutic approach to supplement physiological angiogenesis in ischemic areas for the treatment of diseases involving ischemic conditions. Historically, attempts have been made to apply therapeutic angiogenesis for the treatment of cardiovascular diseases characterized by hypovascularization. These experiences might help to develop therapeutic approaches based on therapeutic angiogenesis for the treatment of pediatric ischemic retinopathy. Coronary artery disease occurs when atheromatous processes prevent blood flow through the coronary artery.³⁰ In contrast, peripheral artery disease is caused by atherosclerotic occlusion of the arteries to the legs.³¹ The ability of various angiogenic factors to induce therapeutic angiogenesis has been tested in preclinical studies and patients with coronary and peripheral artery diseases. The list of these factors includes angiogenin, angiopoietin, FGF, granulocyte colony-stimulating factor, HGF, insulin-like growth factor-1, nitric oxide, platelet-derived growth factor, transforming growth factor, tumor necrosis factor alpha, and VEGF.^{1,32-34} Among them, VEGF, FGF, and HGF have been the most widely studied in clinical trials.

1. Coronary artery disease

Selected clinical studies of therapeutic angiogenesis on coronary artery disease are summarized in **Table 1**, which includes the first trials of different therapeutic materials, phases, and administration methods. These studies exemplify the primary concerns regarding the clinical application of therapeutic angiogenesis for the treatment of human diseases.

Protein therapy

The first clinical trial using a recombinant protein for therapeutic angiogenesis in patients with coronary artery disease tested the therapeutic potential of FGF-1 injected into the myocardium.³⁵ During an elective bypass operation for multivessel coronary artery disease, FGF-1 was applied to the myocardium. In this study, at 12 weeks after the injection, intra-arterial digital subtraction angiography demonstrated that a dense capillary network appeared around the injection site.³⁵ Laham et al.³⁶ showed that there was a trend toward a reduction in the target ischemic area on magnetic resonance assessment in patients who

Table 1. Selected clinical studies on therapeutic angiogenesis for coronary artery diseases

Therapeutic approach	Phase of clinical trials	Administration route	Reference
Protein therapy			
FGF-1	1	Intramyocardial	Schumacher et al. ³⁵
FGF-2	1	Epicardial	Laham et al. ³⁶
VEGF ₁₆₅	1	Intracoronary	Hendel et al. ³⁷
FGF-2 (FIRST trial)	2	Intracoronary	Simons et al. ³⁸
VEGF ₁₆₅ (VIVA trial)	2	Intracoronary	Henry et al. ³⁹
Gene therapy			
Plasmid encoding VEGF ₁₆₅ gene	1	Intramyocardial	Losordo et al. ⁴¹
Adenoviral vector encoding VEGF ₁₂₁ gene	1	Intramyocardial	Rosengart et al. ⁴²
Plasmid encoding VEGF-2 gene	1	Intramyocardial	Vale et al. ⁴³
Plasmid encoding VEGF-2 gene	1/2	Intramyocardial	Losordo et al. ⁴⁴
Adenoviral vector encoding FGF-4 gene (AGENT trial)	1/2	Intracoronary	Grines et al. ⁴⁵
Cell therapy			
BM or circulating blood-derived progenitor cells (TOPCARE-AMI trial)	1	Intracoronary	Assmus et al. ⁴⁸
BM-derived mononuclear cells	2	Intracoronary	Strauer et al. ⁴⁹
BM-derived mononuclear cells	1	Transendocardial	Perin et al. ⁵⁰
BM-derived mononuclear cells	1	Intramyocardial	Tse et al. ⁵¹

FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor; BM, bone marrow.

received an epicardial injection of FGF-2 while undergoing coronary bypass surgery. The first clinical trial using intracoronary recombinant VEGF reported that there was an improvement in myocardial perfusion on single-photon emission computed tomography (SPECT) in patients with severe coronary artery disease who were not optimal candidates for angioplasty or bypass surgery.³⁷ The results of these phase 1 trials were promising. However, the following phase 2 trials failed to provide clear-cut evidence of therapeutic effectiveness for more extensive clinical utilization of therapeutic angiogenesis to treat coronary artery disease.^{3,38-40} A single intracoronary infusion of FGF-2 did not improve exercise tolerance or myocardial perfusion in patients with coronary artery disease who were considered suboptimal candidates for standard surgical or catheter-based revascularization in the FIRST trial.³⁸ In addition, an intracoronary infusion of VEGF did not offer any improvement in exercise treadmill test time by day 60 in patients with stable angina who were judged unsuitable for revascularization based on coronary angiography.³⁹

Gene therapy

In the treatment of coronary artery disease, naked plasmid DNA encoding the VEGF₁₆₅ gene was injected directly into the ischemic myocardium, improving myocardial perfusion on SPECT imaging.⁴¹ Additionally, intramyocardial delivery of an adenoviral vector encoding the VEGF₁₂₁ gene improved symptoms, treadmill exercise assessment, and angiographic assessment in patients with reversible left ventricular ischemia by dobutamine stress echocardiography.⁴² Similarly, intramyocardially administered naked plasmid DNA encoding the VEGF-2 gene also reduced ischemia on electromechanical mapping and improved myocardial perfusion on SPECT scanning.⁴³ A phase 1/2 trial showed that the myocardial transfer of plasmid DNA encoding the VEGF-2 gene through catheter delivery significantly reduced the anginal class in patients with Canadian Cardiovascular Society (CCS) class III or IV angina refractory to maximum medical therapy, multivessel coronary artery disease not suitable for bypass surgery or angioplasty, and reversible ischemia on stress SPECT imaging.⁴⁴ However, another phase 1/2 study (the AGENT trial) on the intracoronary administration of an adenoviral vector encoding the FGF-4 gene only demonstrated insignificant trends of improvement in exercise time in patients with CCS class 2 or 3 angina.⁴⁵

Progenitor cells

Progenitor cells can home to local injured and ischemic tissues and participate in damage repairing and wound healing by secreting growth factors and stimulating neovascularization.^{46,47} In this context, bone marrow-derived or circulating blood-derived cells were administered to patients with coronary artery disease in several clinical trials. In the TOPCARE-AMI trial, an intracoronary infusion of bone marrow or circulating blood-derived progenitor cells was associated with functional recovery at a 4-month follow-up in patients with acute myocardial infarction.⁴⁸ Strauer et al.⁴⁹ attributed the therapeutic effects of intracoronary transplantation of bone marrow-derived mononuclear cells to myocardial regeneration and neovascularization in patients with acute myocardial infarction after mechanical angioplasty and subsequent stent implantation. Transendocardial and intramyocardial implantation of bone marrow-derived mononuclear cells also demonstrated beneficial effects on myocardial blood flow and ventricular function.^{50,51}

2. Peripheral artery disease

Selected clinical studies of therapeutic angiogenesis for peripheral artery disease are summarized in **Table 2**, which includes the first trials of various therapeutic materials, phases, and administration methods.

Protein therapy

In patients with peripheral artery disease and intermittent claudication, FGF-2 was infused into the femoral artery of the ischemic leg in a phase 1 trial by Lazarous et al.⁵² Intraarterial FGF-2 was well-tolerated and increased the blood flow of the calf.⁵² In a phase 2 clinical trial of 190 patients with intermittent claudication, intraarterial FGF-2 resulted in a significant increase in peak walking time at 90 days.⁵³

Gene therapy

The potential of gene therapy in the treatment of peripheral artery disease has been more extensively investigated in several clinical trials. The first clinical trial using a plasmid encoding the VEGF₁₆₅ gene was done in a female patient with 40% stenosis of the proximal popliteal artery on arteriography.⁵⁴ Arterial gene transfer with a hydrogel-coated balloon-angioplasty catheter resulted in an increase in collateral vessels at the knee, mid-tibial, and ankle levels at 4 weeks after treatment.⁵⁴ Intramuscular administration of naked plasmid DNA encoding the VEGF₁₆₅ gene transiently increased serum levels of VEGF, induced

Table 2. Selected clinical studies of therapeutic angiogenesis for peripheral artery diseases

Therapeutic approach	Phase of clinical trials	Administration route	Reference
<i>Protein therapy</i>			
FGF-2	1	Intraarterial	Lazarous et al. ⁵²
FGF-2 (TRAFFIC trial)	2	Intraarterial	Lederman et al. ⁵³
<i>Gene therapy</i>			
Plasmid encoding VEGF ₁₆₅ gene	1	Intraarterial	Isner et al. ⁵⁴
Plasmid encoding VEGF ₁₆₅ gene	1	Intramuscular	Baumgartner et al. ⁵⁵
Plasmid encoding HGF gene	1/2	Intramuscular	Powell et al. ⁵⁶
Adenoviral vector or plasmid encoding VEGF ₁₆₅ gene	2	Intraarterial	Mäkinen et al. ⁵⁷
Adenoviral vector encoding VEGF ₁₂₁ gene (RAVE trial)	2	Intramuscular	Rajagopalan et al. ⁵⁸
<i>Cell therapy</i>			
BM-derived mononuclear cells	1	Intramuscular	Tateishi-Yuyama et al. ⁵⁹

FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor; HGF, hepatocyte growth factor; BM, bone marrow.

newly visible collateral blood vessels on contrast angiography, and improved distal flow on magnetic resonance angiography in patients with nonhealing ischemic ulcers and/or rest pain due to peripheral artery disease.⁵⁵ In a phase 1/2 trial using a plasmid encoding the *HGF* gene, intramuscular injection induced a dose-dependent increase in transcutaneous oxygen tension in patients with critical limb ischemia.⁵⁶ In a randomized, placebo-controlled, double-blinded phase 2 study, intraarterial gene transfer via an adenoviral vector or a plasmid encoding the *VEGF₁₆₅* gene increased vascularity distal to the gene transfer site on digital subtraction angiography in patients with claudication or critical lower-limb ischemia.⁵⁷ Unfortunately, intramuscular delivery of an adenoviral vector encoding the *VEGF₁₂₁* gene did not increase the peak walking time, ankle-brachial index, or quality-of-time measures in another phase 2 randomized, double-blind clinical trial (the RAVE trial) in patients with disabling intermittent claudication.⁵⁸

Cell therapy

As preclinical studies have demonstrated that bone marrow-derived mononuclear cells increased collateral vessel formation in ischemic limbs, intramuscular injection of these cells into the gastrocnemius of patients with unilateral ischemia of the leg improved the ankle-brachial index, transcutaneous oxygen pressure, rest pain, and pain-free walking time.⁵⁹

3. Issues in the application of therapeutic angiogenesis for cardiovascular diseases

Several concerns have blocked the widespread clinical application of therapeutic angiogenesis for cardiovascular diseases. The first concern is whether new capillaries (formed through angiogenesis) without the simultaneous formation of larger arteries (through arteriogenesis) for supplying the capillaries are of less importance and patency.^{60,61} Second, there was no clear consensus on the concentration, the timing, and the area of locally administered angiogenic factors.^{3,33,60} Prolonged tissue exposure to growth factors might be required for the development of robust and sustained neovascularization to secure the survival of the newly formed vasculature.^{62,63} Third, another major concern is excessive vessel growth in the target tissue in the form of hemangioma or a glomeruloid body.⁶⁴ Fourth, concerns have been raised regarding the development of abnormal vessels in other organs after systemic administration of angiogenic factors.^{60,65} In addition, a combination of 2 or more angiogenic factors might be required for functionally mature neovascularization and consistent clinical benefits.⁶⁶⁻⁶⁸ In this context, using different factors acting through complementary mechanisms has been proposed as a solution to the clinical failure of single agents in cardiovascular diseases.⁶⁹ For therapeutic angiogenesis to be applied to the treatment of human diseases, including ischemic retinopathy, these issues should be appropriately addressed.

POTENTIAL OPTIONS OF THERAPEUTIC ANGIOGENESIS AGAINST ISCHEMIC RETINOPATHY

The eye is easily accessible for the local delivery of therapeutic materials using various administration routes, including intravitreal and suprachoroidal injections, which are currently utilized for the treatment of retinal diseases (**Fig. 2**). Antibodies (bevacizumab), antibody fragments (ranibizumab; Genentech), and an antibody-mimicking fusion protein (aflibercept; Regeneron, Tarrytown, NY, USA) are widely administered via intravitreal injections to treat age-related macular degeneration and diabetic retinopathy. In addition,

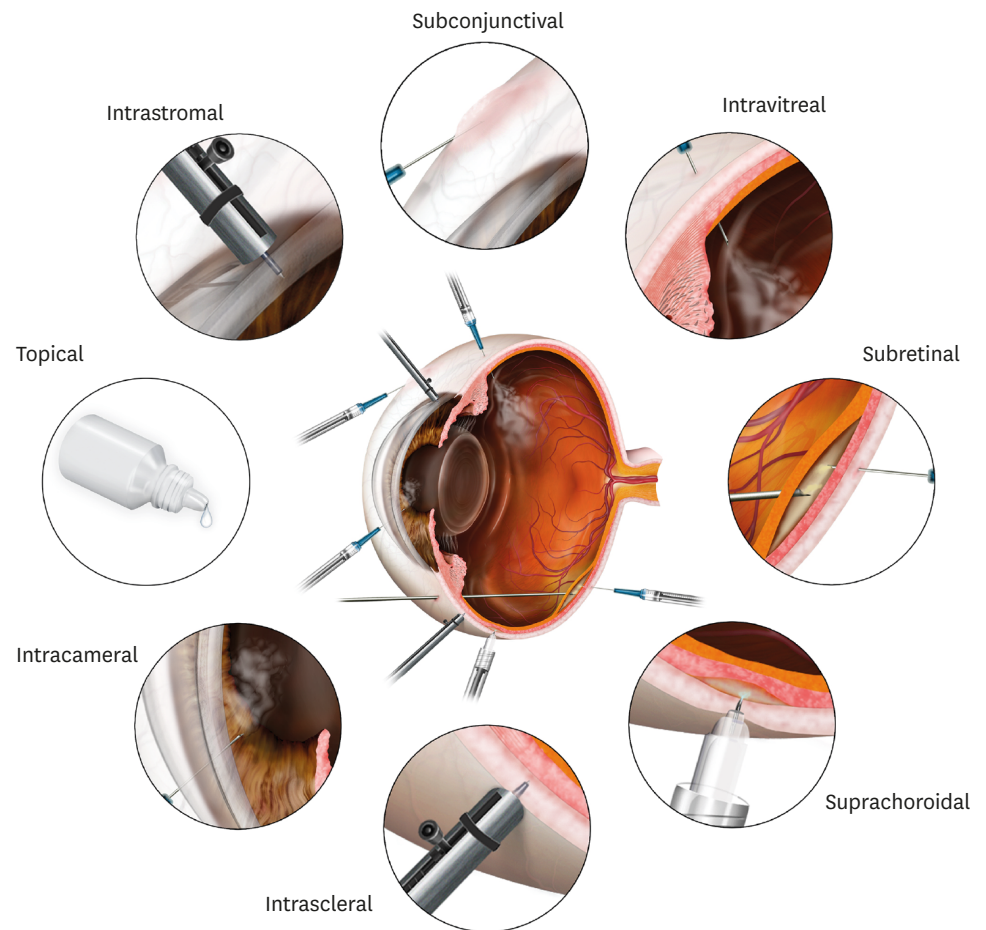


Fig. 2. Various administration routes for delivery of therapeutic materials to the eye. The subretinal and intravitreal routes are currently utilized for the treatment of retinal diseases.

retinal pigment epithelial cells from induced pluripotent stem cells and adeno-associated viral vectors containing therapeutic genes are injected into the subretinal space of patients with age-related macular degeneration and retinal dystrophies, respectively.^{70,71} These administration routes can also be used for therapeutic approaches inducing therapeutic angiogenesis for ischemic retinopathy. Potential options and examples of therapeutic angiogenesis against ischemic retinopathy are summarized in **Table 3**.

For protein therapy, recombinant proteins can be injected into the vitreous cavity. However, VEGF and FGF are unlikely to be suitable for the treatment of ischemic retinopathy via intravitreal injections, because VEGF and FGF levels are usually elevated in the vitreous in patients with ischemic retinopathy.⁷²⁻⁷⁵ Instead, a still-unidentified 'X' protein might be associated with an increase in therapeutic angiogenesis, as opposed to pathological angiogenesis.

One of the candidates for this 'X' protein is norrin, which is encoded by the *NDP* gene. As mentioned, *Ndp*-deficient mice demonstrate a distinct failure in the development of retinal vasculature.^{15,76} It is remarkable that the transgenic expression of norrin by a lens-specific promoter restores the formation of a normal retinal vasculature in *Ndp*-deficient mice.⁷⁶ This implies that intravitreal injection of norrin or ectopic expression of the *NDP* gene

Table 3. Potential options and examples of therapeutic angiogenesis against ischemic retinopathy

Potential therapeutic options and examples	Administration route	Reference
Protein therapy		
Angiopoietin-1	Intravitreal	Lee et al. ⁸¹
Norrin	Intravitreal	Tokunaga et al. ⁷⁸
Gene therapy		
<i>NDP</i>	Transgenic expression	Ohlmann et al. ^{76,77}
Cell therapy		
BM-derived CD34+ cells and vascular wall-derived ECFCs	Intravitreal	Li Calzi et al. ⁸⁸
Circulating CD34+ cells	Intravitreal	Caballero et al. ⁸⁴
Cord blood-derived EPCs	Intravitreal	Wang et al. ⁸⁷
ECFCs from iPSCs	Intravitreal	Prasain et al. ⁸⁶
ECFCs from PBMCs	Intravitreal	Medina et al. ⁸⁵ ; O'Leary et al. ⁹¹

BM, bone marrow; ECFC, endothelial colony-forming cell; EPC, endothelial progenitor cell; iPSC, induced pluripotent stem cells; PBMC, peripheral blood mononuclear cell.

might restore physiological angiogenesis in ischemic retinopathy. Transgenic expression of norrin in the lens or retinal pigment epithelium successfully suppressed the pathological phenotypes of OIR in mice, the most widely utilized animal model of ischemic retinopathy.⁷⁷ In that study, there was no increase in pathological neovascularization. Norrin also decreased the avascular area and inhibited the formation of neovascular tuft in a murine model of OIR.⁷⁸ This effect might be linked with the induction of insulin-like growth factor-1.⁷⁹ Direct injection of the norrin protein or gene delivery through adeno-associated viral vectors of the *NDP* gene might have potential for the treatment of ischemic retinopathy. In another study using COMP-Ang1, a soluble and stable variant of angiopoietin-1,⁸⁰ intravitreal injection of COMP-Ang1 promoted the formation of a vascular network in the central avascular area in OIR mice.⁸¹

Studies on animal models of ischemia provide evidence that endothelial progenitor cells (EPCs) migrate to the ischemic tissue.^{46,47} Circulating progenitor cells expressing the surface marker CD34, which are capable of differentiating into endothelial cells, are recruited to ischemic sites and committed to forming capillaries by hypoxia-regulated factors, such as stromal-derived factor-1, insulin-like growth factor binding protein-3, and VEGF.^{82,83} This tendency can also be utilized in the treatment of ischemic retinopathy. Intravitreally administered CD34+ EPCs incorporate into the damaged retinal vasculature in mice with OIR, after ischemia/reperfusion injury, or streptozotocin-induced diabetic retinopathy, as well as in BBZDR/WOR rats in a rat model of diabetic retinopathy.⁸⁴ Several groups have also reported the therapeutic potential of EPCs, although the protocols and the levels of commitment vary.⁸⁵⁻⁸⁹ Medina et al. reported that outgrowth endothelial cells, also called endothelial colony-forming cells,⁹⁰ with higher expression of CD105 or CD146 from peripheral blood mononuclear cells contributed to vascular repair and reduced the stimuli for pathological angiogenesis.⁸⁵ Prasain et al.⁸⁶ demonstrated that outgrowth endothelial cells from human induced pluripotent stem cells reduced the avascular area and preretinal neovascular tufts. In another study, cord blood-derived EPCs were effective in restoring pathological changes in mice with OIR.⁸⁷ A combination of bone marrow-derived CD34+ cells and vascular wall-derived endothelial colony-forming cells⁸⁸ or co-administration of a peptide based on the helix-B domain of erythropoietin⁹¹ was suggested to enhance the therapeutic effects of EPCs in OIR mice.

CONCLUSION

It is essential to restore the processes of physiological angiogenesis for the direct treatment of patients with ischemic retinopathy. Current treatment options, including laser photocoagulation and surgery, only target the resultant pathological angiogenesis and complications. In the clinical application of therapeutic angiogenesis for human diseases, the most important aspect is the tight regulation of angiogenic processes, from the initiation of angiogenesis to the remodeling of newly formed vessels. The angiogenic process must be controlled in order to obtain a functional vascular network.⁹² Avascular retinal tissues should be exposed to angiogenic factors or progenitor cells for a prolonged time to promote the sustained development of vessels. Preclinical studies using presumptive therapeutic approaches have shown potential, but more studies should be performed for clinical applications to be viable. However, we expect that the clinical application of therapeutic angiogenesis will be more promising in the context of ischemic retinopathy than for diseases in other organs because of the easy accessibility of the eye for local delivery methods. In addition, as in cardiovascular diseases, there is a tremendous unmet clinical need for the development of therapeutic approaches based on therapeutic angiogenesis in the treatment of ischemic retinopathy, as there is no effective pharmacological treatment.^{3,32} We suggest that robust preclinical and clinical studies should investigate the use of therapeutic angiogenesis for the treatment of ischemic retinopathy.

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