The Role of Angiopoietins in Neovascular Diabetes-Related Retinal Diseases

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Received: September 14, 2022 / Accepted: October 14, 2022 / Published online: November 4, 2022 © The Author(s) 2022

ABSTRACT

Diabetic retinopathy is a devastating and frequent complication of poorly controlled diabetes, whose pathogenesis is still only partially understood. Advances in basic research over the last two decades have led to the discovery of angiopoietins, proteins that strongly influence the growth and integrity of blood vessels in many vascular beds, with particular importance in the retina. Angiopoietin 1 (Ang1), produced mostly by pericytes and platelets, and angiopoietin 2 (Ang2), produced mainly by endothelial cells, bind to the same receptor (Tie2), but exert opposing effects on target cells. Ang1 maintains the stability of the mature vasculature, while Ang2 promotes vessel wall destabilization and disruption of the connections between endothelial cells and pericytes. Human retinal endothelial cells exposed to Ang2 show reduced membrane expression of the adhesion molecule VE-cadherin, and patients with proliferative diabetic retinopathy or diabetic macular edema have markedly increased vitreal concentrations of Ang2. Faricimab, a bi-specific antibody simultaneously directed against Ang2 and VEGF, has shown promising results in clinical trials among patients with diabetic retinopathy, and other agents targeting the angiopoietin system are currently in development.

Keywords: Diabetes complications; Retinopathy; Macular edema; Angiopoietins; Tie2; Faricimab

Key Summary Points

Angiopoietins are proteins that modulate blood vessel growth and integrity in various vascular beds.

Ang1 and Ang2 bind to the same receptor (Tie2), but exert contrasting physiological effects.

Ang1 maintains vascular integrity and stability, while Ang2 promotes destabilization.
Patients with proliferative diabetic retinopathy or diabetic macular edema have very high vitreal Ang2 concentrations.

Faricimab is a bi-specific antibody against Ang2 and VEGF, with promising results in proliferative diabetic retinal diseases.

INTRODUCTION

Several retinal diseases, particularly diabetic retinopathy and age-related macular degeneration, are characterized by disruption of the vascular structure and function, which leads to abnormal vascular proliferation. Among the many humoral regulators of new vessel development, a group that has attracted much attention recently is angiopoietins. Angiopoietins were discovered by Davis et al. in 1996 while searching for ligands of the TIE receptor [1], and their involvement in vasculature integrity was promptly demonstrated using transgenic murine models [2]. The purpose of this review is to summarize the current knowledge about angiopoietins, their involvement in the pathogenesis of neovascular diseases of diabetes, and the place that angiopoietin-targeted therapies hold in current pharmacotherapy.

METHODS

We searched Medline, EMBASE, the Cochrane Database of Systematic Reviews and clinicaltrials.gov from inception until October 2022 for the terms “angiopoietin”, “Tie-2”, “diabetic retinopathy”, “macular edema”, “faricimab”, “angiogenesis”, and their Boolean combinations. After an initial screen of the retrieved titles/abstracts for relevance and pertinence, the authors read the full texts of the original papers and extracted key concepts and relevant data. Finally, all five authors were in charge of devising the general structure of the review, as well as writing the manuscript and critically reviewing it.

This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

Angiopoietins and Their Receptor

Angiopoietins can be defined as growth factors that control blood vessel development, repair, and stability. There are four distinct angiopoietins, but the two most exhaustively studied isoforms are Ang1 and Ang2 [3]. These are glycoproteins with a molecular weight of 70 kDa for Ang1 and 57 kDa for Ang2. Both can be found as dimers, trimers, and tetramers, but Ang1 also assembles into higher-order multimers via its N-terminal super-clustering domain (SCD). When Ang1 and Ang2 are in their monomeric form, they are structurally very similar, and share about 60% of their amino acid sequences [4]. Both angiopoietins have a general structure composed of three domains: the aforementioned SCD, a central coiled-coil domain (CCD) that facilitates the asymmetrical dimerization of monomers, and a C-terminal fibrinogen-related domain (FReD), which is required for binding to the Tie2 receptor [5].

Ang1 is predominantly made and secreted by some pericytes and platelets [6], while Ang2 is produced and stored by endothelial cells in Weibel–Palade bodies, before its release into circulation [7]. Ang2 expression increases in response to stimulation by vascular endothelial growth factor (VEGF) [8] or, during inflammation, in response to inflammatory cytokines [9]. It is also upregulated under hypoxia, hyperglycemia, and oxidative stress [10].

Angiopoietins are a demonstrated ligand for the Tie family of receptors. The acronym Tie stands for Tyrosine kinase with Immunoglobulin and Epidermal growth factor homology domains. Tie1 and Tie2 are transmembrane receptors expressed on endothelial cells and tumor-associated macrophages. Both of these are essential for vascular maturation during developmental, physiological, and pathological...
angiogenesis. All angiopoietins are able to bind Tie2, whereas Tie1 is still considered an orphan receptor. Nonetheless, it has been proven that Tie1 is able to heterodimerize with Tie2, and modulate its signal transduction activity [11].

To activate Tie2, angiopoietins bind to the second Ig-like domain of the receptor, a motif that may be present in both Tie2 monomers or Tie1/Tie2 heterodimers [12] (Fig. 1). Only tetrameric or higher multimeric forms of Ang1 induce Tie2 phosphorylation, and in fact, the majority of Ang1 molecules exist in trimeric, tetrameric, and pentameric forms [4]. Meanwhile, Ang2 is rarely found in higher-order forms, and dimers are effective at activating the receptor. This may explain the weaker agonistic potential of Ang2 relative to Ang1 [5]. Of note, Ang1, and to a lesser degree Ang2, induce the formation of Tie2 clusters at endothelial cell–endothelial cell (EC–EC) junctions and endothelial cell–extracellular matrix (ECM) contacts [13]. Tie2 activation is negatively regulated by dephosphorylation by vascular endothelial protein tyrosine phosphatase (VE-PTP) [14].

Ang1 and Ang2 exert contrasting actions. Ang1 is important in maintaining the stability of the mature vasculature. On the other hand, Ang2 promotes vessel wall destabilization by either interacting with integrins, or by competitively inhibiting binding of Ang1 to Tie2. The biological effects of Ang2 appear to be dependent on concomitant VEGF levels: It leads to vessel regression when VEGF is low or absent, but stimulates angiogenesis when VEGF is high [15]. In addition, Ang2 promotes disruption of the connections between the endothelium and pericytes [16].

Binding of Ang1 to Tie2 activates the PI3K and NADPH oxidase signaling pathways, resulting in reorganization of the actin cytoskeleton and accumulation of VE-cadherin at inter-endothelial junctions, both of which strengthen the endothelial barrier [17].

**Role of Angiopoietins in the Pathophysiology of Diabetic Retinopathy**

Angiopoietin dysregulation has been linked to diabetic retinopathy, as angiopoietins regulate the permeability of the blood–retinal barrier and the function of retinal pericytes. Increased concentrations of Ang2 have frequently been found in the vitreous of patients with proliferative diabetic retinopathy (PDR) and diabetic macular edema (DME) [18]. This suggests that, in the context of these diseases, Ang2 most likely inhibits Tie2 activity promoting neovascularization and vascular hyperpermeability [19]. Murine models of DR show an increase in Ang2 mRNA in retinal tissues and an associated decrease in VE-cadherin levels compared to normal controls [20]. In addition, Ang2 activates endothelial expression of beta-1 integrins, leading to vessel destabilization, increased proapoptotic BAX, and decreased anti-apoptotic BCL-2. Interesting recent evidence suggests that Ang2 acts on angiogenesis regulation...
differentially through the Tie2 pathway and integrin signaling [21]. Ang2 binds endothelial beta1-integrin and induces angiogenesis via FAK and RAC-1 activation. In pericytes, meanwhile, stimulation by Ang2 induces apoptosis and, consequently, a reduction in capillary coverage. Most likely, this effect is mediated by Ang2 binding to alpha3/beta1-integrins and signaling through the p53 pathway.

In patients with diabetes, mean plasma Ang2 is higher than in normoglycemic controls, and is positively correlated with HbA1c [22]. In vitro exposure of human retinal endothelial cells to hyperglycemia induces significant increases in Ang2 mRNA and protein, which in turn stimulates phosphorylation and degradation of VE-cadherin, resulting in increased vascular permeability [20] and contributing to macular edema. Even more relevant to diabetic retinopathy, HbA1c correlates not only with the circulating protein but also with intravitreal concentrations of Ang2 [23]. The increase in vitreous levels of Ang2 seems to be specific of active diabetic retinopathy, they are not only up to 15-fold higher than in patients without diabetes, but also about eightfold higher than in patients with inactive diabetic retinopathy [18].

On the other hand, Ang1 upregulates orderly angiogenic processes and favors tightening of endothelial cell junctions, which can ultimately counterbalance the effects of VEGF [24]. VEGF activates Src-mediated vascular endothelial cadherin (VE-cadherin) phosphorylation and internalization, which favors endothelial cell permeability [25]. It is thought that by binding to Tie2, Ang1 could impede the activity of Src, thus halting the increase in vascular permeability [26]. Also, evidence suggests that Ang1 acts through vascular endothelial phosphotyrosine (VE-PTP) (Fig. 2). VE-PTP works as a transmembrane binding partner of VE-cadherin and reduces tyrosine phosphorylation of VE-cadherin. Consequently, the dissociation of VE-PTP and VE-cadherin leads to VE-cadherin phosphorylation and internalization. In this manner, Ang1 promotes the formation of Tie2/VE-PTP/VE-cadherin complexes, preventing vascular hyperpermeability [12].

Multiple studies have explored the influence of angiopoietins on vascular function employing different models and approaches. A study in a rat cardiac allograft atherosclerosis model found that rejected grafts were characterized by very low expression of Ang1 [27], while intracoronary perfusion of cardiac allografts with an adenoviral vector encoding human Ang1 protected against allograft atherosclerosis, inflammation, and fibrosis. Further, endothelial cells from the border zone of an induced myocardial infarction in mice express high levels of Ang2, which promotes multiple phenotypic characteristics of dysfunctional, leaky vessels in this area [28]. In addition to these detrimental effects on vascular integrity, Ang2 from endothelial cells elicited or exacerbated components of the local inflammatory reaction including proinflammatory macrophage polarization, neutrophil infiltration and

![Signaling pathway of the Tie2 receptor.](image)
degradation of the extracellular matrix. Use of an antibody targeted to Ang2 ameliorated all these alterations and the ensuing myocardial remodeling. Thus, Ang2 possesses not only vessel-stabilizing but also strong pro-inflammatory properties.

The involvement of Ang2 in inflammation and immune responses is revealed by the expression of Tie2 in a subset of monocytes/macrophages, in which it induces secretion of IL-10 and Tregs expansion [29]. Ang2 is also able to recruit Tie2(+) monocytes to inflammation or tumor sites. An increased expression of Ang2 has been documented in animal models of systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, systemic sclerosis, and psoriasis [29].

In the IABP-SHOCK II-trial [30], Ang2 levels were measured in 189 patients with a myocardial infarction and cardiogenic shock who received, or did not receive, intra-aortic balloon pump therapy. Higher levels of circulating Ang2 3 days after the onset of cardiogenic shock were associated with greater mortality 1 month and 1 year later (HR 5.15 [2.80–9.45] and HR 5.24 [3.19–8.58], respectively). Immunohistochemical analysis of post-mortem brain specimens from patients who died of a stroke has revealed a greatly enhanced expression of Ang2 in brain vessels from the infarcted zone compared to normally appearing brain tissue [31]. Nonetheless, the association between Ang2 and adverse cardiovascular outcomes is not restricted to the acute scenario. In a recent unbiased analysis of 49 potential biomarkers in samples from patients with heart failure and preserved ejection fraction who participated in the TOPCAT (Treatment of Preserved Cardiac Function Heart Failure With an Aldosterone Antagonist) study, Ang2 was one of the biomarkers with the ability to predict risk of death or hospitalization from heart failure over 3.3 years [32].

**Faricimab**

Faricimab is a bi-specific antibody (bsAb), directed simultaneously against Ang2 and VEGF. The agent is a humanized IgG molecule in which one Fab fragment is directed against Ang2, while the other is directed against VEGF. Both Fab fragments are linked to a common Fc fragment [36, 37] (Fig. 3). The introduction of a P329G mutation in the Fc region prevents the recruitment of immune cells through the Fc gamma receptors [35]. As explained above, Ang2 and VEGF have synergistic mechanisms stimulating neovascularization. In this context, faricimab acts by binding, neutralizing, and depleting both human VEGF-A and Ang2, without neutralizing Ang1 [38]. Thus, faricimab reestablishes the Ang1/Ang2 ratio, favoring vascular stability and integrity.

In a phase I clinical trial in patients with neovascular, age-related macular degeneration (nAMD), faricimab showed a good tolerability and safety profile [39]. Three phase II trials have evaluated faricimab in neovascular diseases: STAIRWAY (NCT03038880 [40]) and AVENUE (NCT02484690, [41]) in patients with nAMD,
and BOULEVARD in patients with diabetic macular edema (DME) (NCT02699450 [42]).

In the STAIRWAY trial, 76 patients with treatment-naive choroidal neovascularization secondary to nAMD and best-corrected visual acuity (BCVA)—Early Treatment Diabetic Retinopathy Study (ETDRS) letter score of 73–24, were randomized 1:2:2 to receive intravitreal ranibizumab 0.5 mg every 4 weeks, faricimab 6 mg every 12 weeks, or faricimab 6 mg every 16 weeks, for 52 weeks (with four initial 4-weekly doses). At week 40, the adjusted mean BCVA gains from baseline were +11.4 (95% CI 7.8–15), +9.3 (6.4–12-3) and +12.5 (9.9–15.1), respectively. At week 52, both faricimab groups resulted in maintenance of initial vision and anatomic improvements comparable to those with monthly ranibizumab [40]. In the AVENUE phase II clinical trial, patients with nAMD were randomized to five different regimens of ranibizumab or faricimab for 36 weeks. At the end of the trial, faricimab did not show superiority in terms of BCVA, but the overall visual and anatomical gains experienced in the faricimab arms supported the pursuit of a phase III clinical trial [41]. The BOULEVARD phase II randomized trial tested the safety and efficacy of faricimab compared to ranibizumab in patients with DME. The trial revealed superior results for faricimab in visual acuity gains, central subfield thickness (CST), Diabetic Retinopathy Severity Scale (DRSS) score, and durability (assessed by time to retreatment) [42].

Recently, four phase III clinical trials evaluating the efficacy of faricimab have been reported: TENAYA (NCT03823287) and LUCERNE (NCT03823300) in nAMD, and YOSEMITE (NCT03622580) and RHINE (NCT03622593) in DME.

TENAYA and LUCERNE were randomized, double-masked, non-inferiority trials that assigned treatment-naive patients with nAMD to either faricimab 6.0 mg up to every 16 weeks, or aflibercept 2.0 mg every 8 weeks. The primary endpoint was mean change in BCVA from baseline, averaged over weeks 48, 52, and 56, using the ETDRS letters. Faricimab every 8 weeks was non-inferior to aflibercept every 8 weeks, mean adjusted changes were respectively 10.7 (9.4–12.0) versus 10.9 letters (9.6–12.2) in YOSEMITE, and 11.8 (10.6–13.0) versus 10.3 (9.1–11.4) letters in RHINE. Faricimab by PTI was also non-inferior to aflibercept every 8 weeks. In summary, faricimab has shown clinically relevant vision gains and anatomical improvements in both nAMD and DME. For this reason, the FDA has recently approved its usage for nAMD and DME under the brand name Vabysmo® [45].

Currently, extension trials are underway for TENAYA and LUCERNE (AVONELLE-X, NCT04777201), as well as for YOSEMITE and RHINE (Rhone-X, NCT04432831). Also, a phase 4 study addressing the efficacy of faricimab in underrepresented treatment naïve DME patients (ELEVATUM-NCT05224102) is currently under way. Patients will be treated with 6-mg intravitreal faricimab injections every 4 weeks for the initial 20 weeks. Then, they will switch to 6-mg intravitreal faricimab every 8 weeks until week 52. Participants will be followed up until week 56, and assessed for change in BCVA relative to baseline.

Table 1 shows the comparative efficacy of faricimab relative to other available therapies for DME.

 Meanwhile, in LUCERNE the vision gains were 6.6 letters for both groups [43]. These results evidenced that faricimab is non-inferior to aflibercept in the treatment of nAMD, with the added benefit of longer between-dose intervals. YOSEMITE and RHINE by contrast involved only patients with DME. Both were double-masked, non-inferiority trials of faricimab versus aflibercept. In total, 1891 patients were randomized (1:1:1) in the two trials to one of three groups: (1) faricimab 6.0 mg every 8 weeks; (2) faricimab 6.0 mg with a personalized treatment interval (PTI); or (3) aflibercept 2.0 mg every 8 weeks [44]. The PTI dosing intervals could be reduced (every 4 weeks), maintained (every 8 weeks), or extended (every 16 weeks), depending on disease activity at active dosing visits. The primary endpoint was mean change in BCVA averaged over weeks 48, 52, and 56, using the ETDRS letters. Faricimab every 8 weeks was non-inferior to aflibercept every 8 weeks, mean adjusted changes were respectively 10.7 (9.4–12.0) versus 10.9 letters (9.6–12.2) in YOSEMITE, and 11.8 (10.6–13.0) versus 10.3 (9.1–11.4) letters in RHINE. Faricimab by PTI was also non-inferior to aflibercept every 8 weeks. In summary, faricimab has shown clinically relevant vision gains and anatomical improvements in both nAMD and DME. For this reason, the FDA has recently approved its usage for nAMD and DME under the brand name Vabysmo® [45].

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Nesvacumab (REGN910)

Nesvacumab is a human immunoglobin G1 (IgG1) monoclonal antibody that selectively binds and blocks the action of Ang2 in the Tie2 receptor. The drug was well tolerated in its phase I clinical trial, where it was prescribed in conjunction with aflibercept in patients with nAMD or DME (NCT01997164). However, in the phase II clinical trials RUBY and ONYX, the combination was not superior to aflibercept monotherapy (NCT02713204, NCT02712008). Thus, Regeneron decided in 2017 to suspend further clinical development of the agent [10].

Razuprotafib (AKB-9778)

Razuprotafib is a small molecule that works as a competitive inhibitor of the catalytic domain of VE-PTP, impeding Tie2 dephosphorylation and enhancing Tie2 signaling [49]. In phase I clinical trials, razuprotafib systemic (subcutaneous) administration for 4 weeks was safe and well tolerated in patients with nAMD or DME (NCT01997164). However, in the phase II clinical trials RUBY and ONYX, the combination was not superior to aflibercept monotherapy (NCT02713204, NCT02712008). Thus, Regeneron decided in 2017 to suspend further clinical development of the agent [10].

AXT107

AXT107 is a small peptide derived from collagen IV that exerts its action by destabilizing the alpha5-beta1 integrin, leading to relocation of Tie2 to endothelial cell junctions and potentiation of Tie2 activation. Under these circumstances, Ang2 action mimics that of Ang1 [51].

Table 1 Summary of the efficacy of currently available therapies for DME

| Treatment                | Dose/frequency                              | Visual acuity gain                                                                 | References |
|--------------------------|---------------------------------------------|-----------------------------------------------------------------------------------|------------|
| Ranibizumab              | 0.5 mg every 4 weeks                        | Between + 11.0 and + 11.4 in BCVA                                                 | [46]       |
| Aflibercept              | 2.0 mg every 4 or 8 weeks                   | Between + 11.1 and + 11.5 in BCVA                                                 | [47]       |
| Ranibizumab + laser      | Ranibizumab 0.5 mg every 4 weeks, focal/grid laser simultaneously or ≥ 4 weeks after ranibizumab initiation | + 7.2 letters in prompt laser and + 9.8 letters in delayed laser                  | [48]       |
| Brolucizumab             | 6.0 mg every 4 weeks                        | + 12.2 in BCVA                                                                      | NCT03917472|
| Faricimab                | 6.0 mg every 8 weeks                        | Between + 10.7 and + 11.8 in BCVA                                                 | [43]       |

(2) Subcutaneous razuprotafib twice daily + monthly intravitreal ranibizumab; and (3) Monthly intravitreal ranibizumab alone. The combination therapy arm showed a significant reduction in the central subfield thickness (a parameter of DME severity), at 12 weeks versus the anti-VEGF monotherapy arm (p = 0.008). In the phase IIb clinical trial, TIME-2b (NCT03197870), patients with moderate-to-severe non-proliferative diabetic retinopathy were assigned to receive razuprotafib 15 mg once daily, razuprotafib 15 mg twice daily, or matching placebo. The primary end point was the percentage of patients who improved by two or more steps in the DRSS during a 48-week period. Unfortunately, the study did not achieve its primary endpoint and was discontinued.
AXT107 is delivered via intravitreal injections. There is an ongoing phase I/IIa clinical trial evaluating the safety and bioactivity of AXT107 at three different dosing schemes in patients with DME (NCT04697758).

CONCLUSIONS

Angiopoietins are essential players in the maintenance of vascular homeostasis and constitute a formidable target for the treatment of diabetic retinopathy and DME. Future studies will establish the role of angiopoietin-based therapeutics in the usual management of these diseases.

ACKNOWLEDGEMENTS

Funding. No funding or sponsorship was received for this study or publication of this article.

Author Contributions. All authors contributed equally to the literature search, extraction and analysis of the information, and writing and critical review of the article.

Disclosures. Juan David Collazos-Alemán has nothing to disclose, Sofia Gnecco-González has nothing to disclose, Beatriz Jaramillo-Zarama has nothing to disclose, Mario A. Jiménez-Mora has nothing to disclose, Carlos O. Mendivil has nothing to disclose.

Compliance with Ethics Guidelines. This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

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