

New Molecular Targets in the Treatment of Ocular Neovascular Diseases

The role of platelet-derived growth factor-B for inhibiting growth of aberrant vessels.

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Over the past decade, treatment strategies for ocular neovascular diseases have progressed from purely ablative laser-based procedures to the development of pharmacological agents that target factors underlying the pathogenesis of aberrant blood vessel growth. The principal focus of recent research effort has been vascular endothelial growth factor A (VEGF-A), which exerts a variety of roles such as endothelial cell mitogen,¹ survival factor² and inducer of vascular permeability,³ and is a key mediator of both physiological and pathological vascularization.^{4,5}

This new focus has already yielded one approved therapy, pegaptanib (Macugen; OSI/Eyetech, New York, NY and Pfizer, New York, NY). This RNA aptamer, which inhibits the 165 isoform of VEGF,⁶ has been proven effective in the treatment of neovascular age-related macular degeneration (AMD),⁷ and has shown promise in the treatment of diabetic macular edema⁸ and central retinal vein occlusion.

VEGF-A ALONE MIGHT NOT BE ENOUGH

Evidence from tumor angiogenesis models, however, demonstrate that inactivation of VEGF-A alone may not be sufficient to cause regression of mature blood vessels.^{9,10} Maturity of vessels is a poorly defined state commonly attributed to the presence of vascular mural cells (pericytes surrounding the capillaries and smooth muscle cells surrounding larger blood vessels). Vascular stability and function are dependent on mural cells and the recruitment of mural cells by endothelial cells to envelop the developing vasculature is activated by platelet-derived growth factor B (PDGF-B) and signaling through the PDGF receptor beta (PDGFR-beta).

The role of PDGF-B and its receptor was established with the use of transgenic mice that lack expression of either PDGF-B or PDGFR-beta.^{11,12} Recruitment of mural cells was significantly inhibited in the absence of PDGF-B or its receptor^{11,12} resulting in endothelial cell hyperplasia and abnormalities in the development and maturation of nascent vasculature.¹³

Localized secretion of VEGF by pericytes is also known to be important for endothelial cell survival. In addition, mural cells that surround tumor vessels have been found to produce VEGF-A1;^{4,15} given their tight apposition to the endothelial cells which they envelop, access by anti-VEGF agents could well be impeded.

Therefore, it is possible that the efficacy of anti-VEGF therapy may be compromised by the presence of mural cells, and that agents that disrupt mural cell recruitment could potentiate the effect of VEGF-targeting agents such as pegaptanib. Interference with both VEGF-A and the signaling pathway activated by PDGF-B may aid in the goal of inhibiting the growth and stability of aberrant vessels in tumor or ocular neovascular disease.

THREE MURINE MODELS

To assess whether depleting mural cells would enhance the effect of anti-VEGF blockade on new vessels and if the blockade would remain effective over time, we evaluated the use of VEGF-A and PDGFR-beta antagonists in combination for the treatment of ocular angiogenesis. This mini-review presents the latest findings of our experiments in three murine models of ocular neovascularization.¹⁶

We first examined the impact of this combined inhibition on the physiological development of reti-

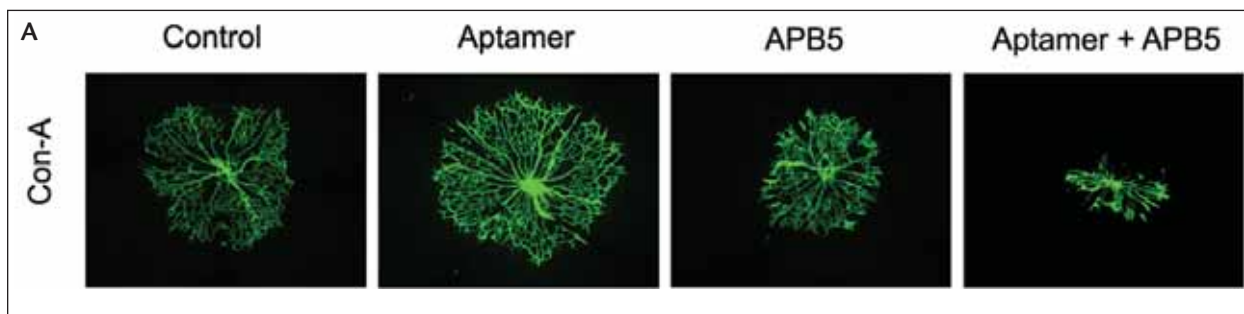
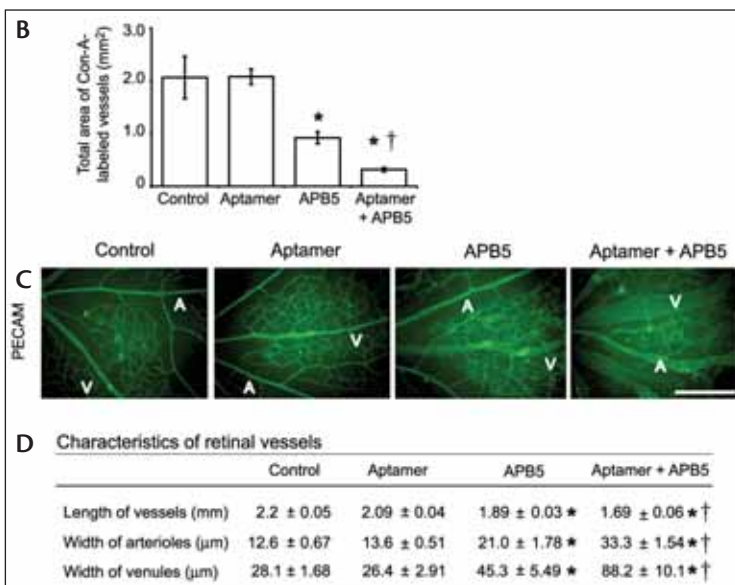


Figure 1. Blocking VEGF-A and PDGF-B signaling affects retinal vascular growth and morphology. Neonatal mice were injected daily with anti-VEGF aptamer or APB5 or both. P3 retinal vessels in each treatment group were labeled by perfusion with concanavalin A (green) (A). Quantification of the total vessel area in the P3 retina (B). P7 retinal vasculature was labeled with PECAM-1 staining. Scale bar, 100 μ m (C). Analysis of P7 retinal vessel length and width. * $P < .01$ compared to control or anti-VEGF aptamer-treated mice; † $P < .05$ compared to APB5-treated mice (D).



nal vasculature in neonates. It has been established that recruitment of pericytes in this context is prevented by inhibition of signaling through PDGFR-beta.¹⁷ We also used 2 models of pathological ocular neovascularization. In one model, choroidal neovascularization (CNV) is induced by laser burns to the retina and reproduces many of the features of AMD. The second, a model in which corneal neovascularization is induced by injury to the corneal epithelium, provides an especially favorable experimental system. The induced blood vessels are easily visualized (as are the impacts of any experimental interventions) and do not generally regress under natural conditions.

For experiments in the three models, PDGF-B signaling was inhibited by systemic exposure to the anti-PDGFR-beta antibody APB5 or imatinib, a small molecule inhibitor of PDGFR-beta kinase activity. VEGF-A signaling was inhibited with systemic administration of pegaptanib (50 mg/kg), while injections of phosphate-buffered saline (PBS) served as control. Blood vessel development was assessed through whole-mount immunofluorescence. Assays were also done to evaluate cell proliferation and apoptosis and to quantify levels of expression of VEGF-A, PDGF-B and PDGFR-beta.

In all three experimental models, dual-inhibition of VEGF-A and PDGF-B signaling was more effective in

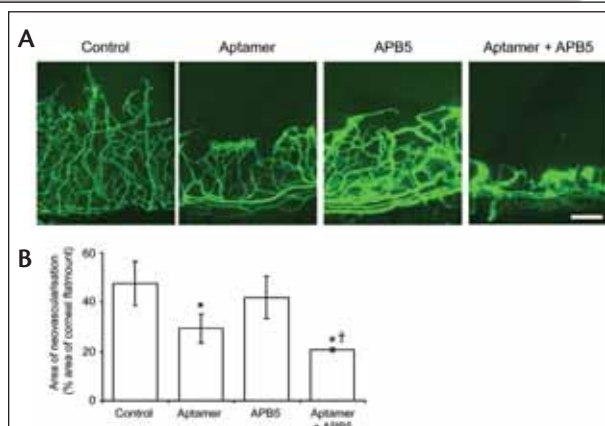


Figure 2. Combination therapy prevents vessel growth in the corneal neovascularization model. Mice were treated daily immediately following corneal neovascularization induction for 10 days with either APB5 or anti-VEGF aptamer or both. Neovascularization was analyzed on day 10 following perfusion with fluorescein isothiocyanate-concanavalin A. Scale bar, 100 μ m (A). Quantitative analysis of corneal area covered with neovascularization. * $P < .01$ compared to control or PDGF-B inhibitor-treated mice; † $P < .05$ compared to anti-VEGF aptamer-treated mice (B).

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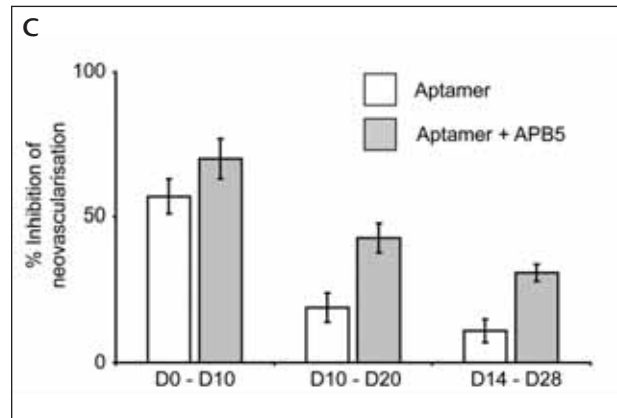
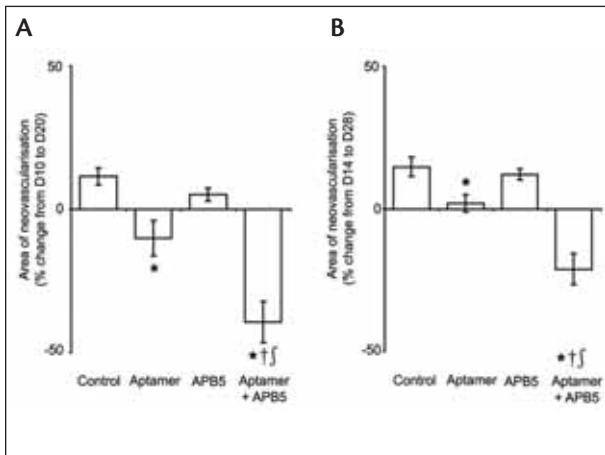


Figure 3. Combination therapy caused vessel regression over time. Treatment of CNV started at 10 days postinjury for 10 days. Quantitative analysis of neovascular regression expressed as the percent change in neovascularization area at day 20 (after 10 days of treatment) compared to day 10 (before treatment; represented as 0 on the y axis). * $P < .05$ compared to day 20 control or PDGF-B inhibitor-treated mice; † $P < .01$ compared to anti-VEGF aptamer-treated mice. $P < .01$ compared to day 10 mice (before treatment) (A). In this experiment treatment was started 14 days postinjury until day 28 (day 14 to day 28). Quantitative analysis of regression expressed as the percentage change in neovascularization area as compared to day 14 (before treatment). * $P < .05$ compared to day 28 control mice; † $P < .01$ compared to anti-VEGF aptamer-treated mice. $P < .01$ compared to day 14 mice (before treatment) (B).

Figure 3 cont'd. The efficacy of the anti-VEGF aptamer diminishes over time. Animals were treated at different times postinjury from day 0 to day 10, day 10 to day 20, or day 20 to day 30. Combination therapy is more effective over time than anti-VEGF inhibition alone (C).

Further analysis indicated that the enlargement of these vessels under the combined treatment reflected changes in endothelial cell shape and organization.

causing vessel regression than blocking VEGF-A alone. Here is a summary of data from these experiments and a discussion of potential implications of these findings.

Developing Neonatal Retinal Vasculature Model

As expected, daily administration of the anti-PDGF-B antibody APB5 from birth prevented mural cell recruitment to developing blood vessels and also led to significant inhibition of retinal blood vessel growth at postnatal day 3. In contrast, pegaptanib did not affect blood vessel development when administered alone, but provided a further reduction in retinal vascularization when administered together with APB5, compared with administration of APB5 alone (Figures 1A and 1B). This activity differs from that reported with nonselective VEGF inhibitors and is likely due to the specificity of the aptamer to a subset of VEGF isoforms.¹⁸ Continued administration of these agents through P7 led to significantly enlarged vessels, in the case of APB5 alone, in keeping with earlier findings by other groups,^{13,17} and still greater vessel enlargement in

animals treated with both APB5 and pegaptanib (Figures 1C and 1D). Further analysis indicated that the enlargement of these vessels under the combined treatment reflected changes in endothelial cell shape and organization, rather than increased proliferation, accompanied by an increase in endothelial cell apoptosis (data not shown).

Corneal Neovascularization Model

In the corneal neovascularization model, blood vessels grew into the injured corneal area for about a week following epithelial debridement, after which time further growth was minimal; by 10 days postinjury, the blood vessels were well-covered with mural cells. This mural cell coverage was greatly reduced by administration of antibody APB5 from days 1 through 10 postinjury (data not shown) but blood vessel growth proceeded essentially unimpeded (Figure 2). In contrast, pegaptanib administered over the same period significantly inhibited blood vessel growth, while combined injections of pegaptanib and APB5 provided an additional reduction over administration of pegaptanib alone (Figure 2).

We also employed the corneal neovascularization

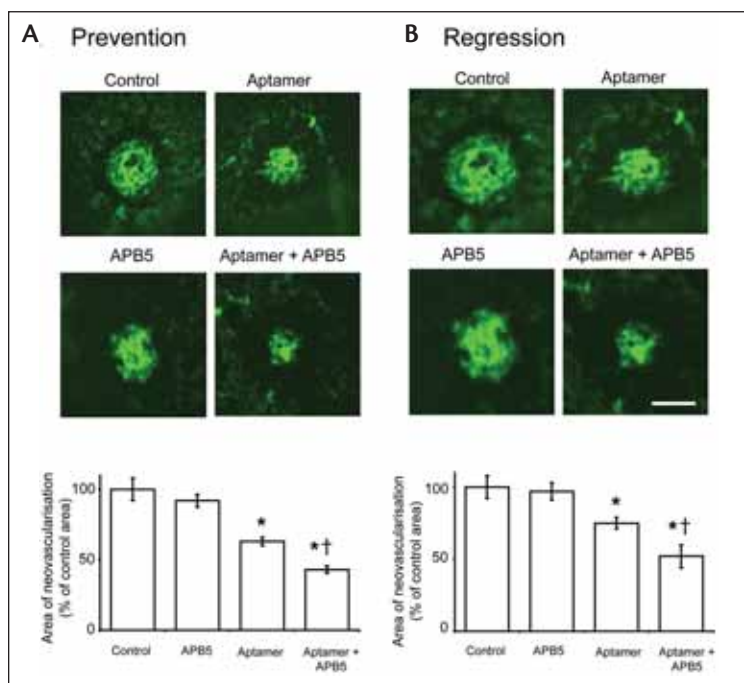


Figure 4. Combination therapy is effective both in prevention and intervention treatments of mouse CNV. **Prevention.** Animals were treated with either APB5 or anti-VEGF aptamer or both for 14 days following laser injury. Representative images of CNV are visualized by PECAM-1 staining. Quantitative analysis of the CNV area. * $P < .01$ compared to control or APB5-treated mice; † $P < .05$ compared to anti-VEGF aptamer-treated mice. **Combination therapy is also effective in treating later stage CNV (A).** **Regression.** Mice were treated with APB5 from 7 days postinjury. At day 14 postinjury, neovascularization was analyzed after labeling vessels with PECAM-1. Quantitative analysis of CNV area. * $P < .01$ compared to control or PDGF-B inhibitor-treated mice; † $P < .05$ compared to anti-VEGF aptamer-treated mice (B).

model to assess the impact of the inhibition of VEGF-A and PDGF-B signaling on the integrity of more established neovasculature. In these experiments, initiation of pegaptanib and/or APB5 treatment was delayed until either day 10 or day 14 (in some of these experiments, imatinib was used in place of APB5, with similar results), with treatment maintained until day 20 and day 28, respectively. As shown in Figure 3A, pegaptanib inhibited vessel growth, compared to day 20 PBS-injected controls, although there was no significant vessel regression.

Corneal neovessels of mice treated with APB5 were stripped of their mural cells, but total neovascular area was similar to that of the day 20 controls, suggesting APB5 alone did not alter vessel growth. Interestingly, mural cells in the normal, quiescent limbal vessels were not removed by APB5, suggesting that only neovessels are affected. Using a combination of both APB5 and

pegaptanib led to a dramatic decrease in new vessel growth compared with PBS-treated controls, and significant regression of neovessels from day 10.

When the same experiment was initiated at day 14, with injections continuing until day 28, qualitatively similar results were seen (Figure 3B), except that the modest regression caused by pegaptanib on its own was no longer observed, suggesting that the maturing blood vessels became progressively more resistant to anti-VEGF therapy, as had been seen in the studies of tumor vascularization.^{9,10} The progressive diminution in pegaptanib efficacy as blood vessels mature is further demonstrated in Figure 3C; note that at all time points there is a greater effect of combined PDGF-B/VEGF-A inhibition than is seen with VEGF-A inhibition alone. Further control experiments confirmed that the decreased efficacy of pegaptanib at later time points was not the result of less pegaptanib being present in the cornea.

CNV Model

The model used in these experiments, in which laser injury to Bruch's membrane is used to initiate CNV, is commonly employed in studies of AMD.¹⁹ We have examined the effects of single and combined inhibition of VEGF-A and PDGF-B signaling in two contexts: prevention of

CNV development and regression of established CNV. For the prevention study, daily injections of APB5, pegaptanib, or both were continued for 14 days postinjury. While APB5 had little effect on the extent of CNV, significant reduction was observed with pegaptanib, and still greater inhibition occurred in mice treated with both agents (Figure 4). In the regression study, in which treatment was delayed until 7 days postinjury, the results were essentially the same.

DISCUSSION

The principal conclusion of this series of experiments involving 3 separate models of ocular vascularization is that concurrent inhibition of PDGF-B and VEGF-A signaling was superior to inhibition of the VEGF-A pathway alone in mature vessels. The combined treatment was able to induce the regression of existing vessels, while inhibition of VEGF-A alone was

not. These data are of particular interest in the context of developing new therapies for ocular neovascular conditions, since patients presenting with conditions such as AMD generally have a mixture of mature and nascent blood vessels in their lesions. Interestingly, results of a subgroup analysis of patients receiving pegaptanib therapy for treatment of AMD suggest that earlier lesions are more susceptible to this intervention.²⁰ Our findings from the corneal neovascularization model demonstrating that pericytes were stripped from newly growing vessels, but not from existing limbal vessels, suggest that the added efficacy that is afforded by combined VEGF-A/PDGF-B inhibition also has a temporal window, but one which is clearly broader than that observed for pegaptanib alone.

An aptamer directed against PDGF-B has recently been successfully employed as an intravitreal agent to inhibit neovascularization in an experimental model.

As to the direct application of this work to the clinic, it is clear that considerable effort is required to further delineate the nature of the efficacy and safety of combined inhibition. Nonetheless, it is safe to say that intravitreal injection offers significant advantages from the perspective of safety²¹ and that aptamer technology, by generating reagents which are inherently nonimmunogenic,²² presents an especially attractive alternative when multiple agents are being administered. In this context, it is of particular interest that an aptamer directed against PDGF-B has recently been successfully employed as an intravitreal agent to inhibit ocular neovascularization in an experimental model system.²³ There are no apparent impediments to combining such reagents with pegaptanib for use in the clinic. ■

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