Combination therapy for ocular and tumor angiogenesis

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This series of studies evaluates the activity of three angiostatic compounds that target various angiogenic processes, including vascular endothelial growth factor (VEGF), extracellular endothelial cell survival, and endothelial intracellular adhesion and lumen formation. The compounds are studied alone and in combination, in animal models of retinal neovascularization (both developmental and pathological), and in tumor vasculature. Angiostatic monotherapy resulted in the upregulation of proangiogenic factors, while combination therapy resulted in significantly less upregulation. Angiostatic combination therapy had synergistic angiostatic and antineovascular effects in retinal models, obliterated tumor vasculature and prolonged survival, and did not affect normal vasculature in any model. These studies point to the utility of combination therapy in the clinic. Even though anti-VEGF therapy is currently the only available angiostatic therapy for ocular choroidal neovascularization, these studies support the concept of combination therapy using agents with different mechanisms of action.


Dorrell and colleagues examine multiple angiostatic agents in animal models for normal retinal vascular development, ischemic retinopathy and tumor growth [1]. They demonstrate that combining angiostatic agents with distinct mechanisms of action synergistically inhibits normal vascular development, pathological neovascularization and tumor growth.

Age-related macular degeneration (AMD), characterized by abnormal choroidal neovascularization (CNV), is a leading cause of blindness in individuals over 50 years of age. Multiple proangiogenic mediators stimulate the formation of CNV. Since the underlying stimulus for CNV is poorly understood, treatments that eliminate pathological neovascularization while maintaining normal angiogenic activity are highly desired. Within the past few years, angiostatic agents that target vascular endothelial growth factor (VEGF) have been introduced to treat patients with CNV due to AMD (i.e., pegaptanib [Macugen®], ranibizumab [Lucentis™], and bevacizumab [Avastin®]). While these agents have produced stable or improved visual acuity [2,3,4], they have limitations, including high cost, rigorous dosing schedule and unknown, but potentially deleterious, effects of long-term VEGF suppression. Thus, many clinicians have begun combining agents in order to improve disease suppression and reduce exposure to antiangiogenic agents compared with monotherapy.

Methods & results

This series of studies compared the effectiveness of three angiostatic compounds with different targets:

- A VEGF aptamer identical to pegaptanib to suppress VEGF165
- A small molecule $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin antagonist to target extracellular matrix-mediated endothelial cell survival
• T2-TrpRS (T2), a proteolytic fragment of tryptophan tRNA synthetase, to block endothelial intracellular adhesion and lumen formation.

The three compounds were evaluated as monotherapies and as combination therapy (two or three of the compounds used together). Effectiveness was tested in three animal models: mouse retinal developmental angiogenesis, mouse ischemic retinopathy and rat gliosarcoma growth.

In the mouse retinal development model, the application of optimum doses of monotherapy (2.0 µg/eye VEGF aptamer; 10 µg/eye integrin antagonist; 0.25 µg/eye T2) produced no angiostatic effect in a third of treated retinas, while only 17–35% of the retinas (depending on the monotherapy) displayed high levels of inhibition. By contrast, the combination of all three angiostatic agents produced over 90% inhibition and only two of 24 treated retinas showed substantial neovascularization. Furthermore, in combination, substantially lower concentrations produced angiostasis compared with the monotherapies.

In the mouse model of oxygen-induced retinopathy, the combination approach again proved beneficial. A combination of any two of the angiostatics displayed significantly less pathological neovascularization than any of monotherapies of the same concentration. Triple therapy also reduced pathological vessel formation.

In the rat gliosarcoma model, triple therapy eradicated tumor vasculature. After intratumoral convection-enhanced delivery of angiostatic triple therapy, the rats developed large, avascular tumor regions with mononuclear cell infiltrate. Four out of seven rats had complete vascular obliteration of the tumor region, while three had regions of normally vascularized tumor growing peripheral to the avascular zone. Tumors in both monotherapy- and control-treated rats displayed typical tumor vasculatization.

To explain their findings, the authors theorized that monotherapies induce a compensatory upregulation of proangiogenic factors. To test the theory, they used enzyme-linked immunoabsorbent assay-based assays to quantify the expression of angiogenic proteins in normal retinas, and control-, monotherapy-, and combination therapy-treated retinas. Their findings reveal that multiple angiogenic pathways are upregulated in response to monotherapy, but not triple therapy. These findings support the hypothesis that upregulation of compensatory factors may prevent single angiostatics from inhibiting neovascularization.

Discussion & significance
Angiogenesis, a complicated process that occurs in many diseases, is dependent upon numerous processes that are linked together – the angiogenic cascade. The cascade begins with an angiogenic stimulus, such as hypoxia or trauma, which results in the synthesis and release of proangiogenic agents, such as VEGF, tumor necrosis factor-α, fibroblastic growth factor-α and -β, and insulin-like growth factor-1. These agents bind to and activate vascular endothelial cells, which leads to matrix metalloproteinase production and release, extracellular matrix degradation and remodeling, and vascular endothelial cell migration and proliferation. Subsequently, this results in lumen formation and vascular formation.

While the authors of the paper under evaluation show that combination therapy inhibits pathologic neovascularization more effectively than monotherapy, more important is the result that an angiostatic monotherapy leads to a strong angiogenic response (upregulation) than a combination of angiostatic treatments. This means that inhibition of different steps of the angiogenic cascade can significantly reduce upregulation of angiogenic factors and may limit or stop disease progression.

The results of Dorrell and colleagues explain why monotherapeutic angiostatic therapy fails to inhibit neovascularization. The synergy of drugs that block the angiogenic cascade at different steps in the pathway is familiar in oncologic medicine, where combination treatment strategies are established. Likewise, in ophthalmology, the concepts that this paper demonstrates have significance in neovascular diseases, including AMD. The results shown in this paper are promising but a long way from direct clinical application. This research highlights the need for development of antiangiogenic agents that manipulate targets other than VEGF, a requirement that was made clear from a recent high-level clinical perspective [5]. This research will be directly applicable only when other potent antiangiogenic compounds are available for use in the clinic.

Expert commentary & conclusion
How do the concepts underscored by this work apply to the retina specialist’s current practice environment? Can combination therapy with currently available drugs be used to overcome the limitations of monotherapies?

Along with the anti-VEGF therapies available to treat CNV due to AMD, as mentioned previously, additional therapies are available that act by different mechanisms of action: verteporfin photodynamic therapy (PDT; Visudyne®), which destroys existing CNV, and anti-inflammatory drugs, such as triamcinolone acetonide (TA) and dexamethasone. These therapies may be combined in double and triple therapies, extending the combination therapy concepts of this research paper beyond purely angiostatic therapies.

As Dorrell and colleagues demonstrate, monotherapeutic approaches in neovascular disease treatment have limitations. While the introduction of anti-VEGF drugs represented a substantial advance in the treatment of AMD, these drugs have limitations. Anti-VEGF drugs reduce vascular permeability and have a modest effect on inhibiting the formation of neovascular vessels. Thus, they reduce edema and improve visual acuity but they do not eradicate the underlying, established CNV [6].

Similarly, monotherapy with verteporfin PDT has its limitations. It eradicates CNV and upregulates the antiangiogenic pigment epithelium-derived factor [7], but it does not adequately address the edema and inflammatory aspects of AMD, and may even enhance them through additional inflammatory upregulation and VEGF expression [7]. However, owing to the potential for post-PDT inflammation, PDT has previously been...
combined with intravitreal TA to reduce the inflammatory reaction. Owing to cataract development and intraocular pressure elevation associated with TA, dexamethasone, a high-potency, short half-life steroid, may also prove advantageous in combination. There are theoretical mechanistic advantages to combining multiple currently available agents for AMD treatment.

The role of combination therapies for AMD is already beginning to emerge clinically [8]. Recent pilot studies have shown the safety and efficacy of combined use of anti-VEGF agents with verteporfin PDT, with or without corticosteroids [9,10]. These studies, as well as emerging clinical trends, suggest that the combined use of these agents produces efficacy comparable with that of anti-VEGF monotherapy, with a markedly decreased treatment burden. For example, Augustin and colleagues report visual acuity results similar to that of anti-VEGF monotherapy (1.8 lines gained) with a low retreatment rate of approximately 20–25%. By contrast, anti-VEGF monotherapy may require retreatment every 4–6 weeks for maintained visual acuity benefit.

While Dorrell and colleagues suggest that combining angiostatic agents is synergistic, in the clinic using the currently available agents, efficacy for anti-VEGF/PDT or anti-VEGF/PDT/corticosteroid combinations appears equivalent with anti-VEGF monotherapy. Further refinement of combination therapy protocols may show additive benefits in the future. The effect of alternative doses (drug doses, as well as light doses for PDT) and treatment timing is largely uncharted and provides opportunities for optimization and efficacy improvements over both monotherapy and combination therapy.

Currently, the chief advantage for patients of existing combination therapy protocols is a reduction of treatment burden. Dorrell and colleagues note the use of relatively low doses of angiostatic agents as a reason for developing combination strategies. Indeed, VEGF is required to maintain normal neurological function and to maintain normal vascular architecture and physiology [6], and the long-term, potentially deleterious effect of chronic VEGF suppression is not known. In the pilot studies of existing therapies used in combination, the reduced number of treatments reduce patient exposure to anti-VEGF agents, as well as reduce costs and the burden of frequent anti-VEGF monotherapy dosing [9,10]. Thus, early clinical combination therapy results display an advantage that is common to the basic science combination therapy results that have been reviewed here, that is, reduced dosing.

Five-year view
Certainly retinal specialists are eager to have more angiostatic therapies available to treat patients with CNV due to AMD. Potential synergistic effects, as demonstrated by Dorrell and colleagues, using lower doses than with current anti-VEGF therapies, would be welcome in clinical practice, both to avoid reflux in intravitreal administration and to minimize the risk of effects on normal vasculature that could theoretically lead to serious events, such as myocardial infarction or stroke. Reports of the lack of clinical development of new angiostatic agents are discouraging [5], but Dorrell and colleagues have made it clear that multiple angiostatic therapies have great clinical potential.

Key issues

- Angiogenesis, a fundamental biological process that is essential to survival, manifests in pathologies, such as choroidal neovascularization (CNV) due to age-related macular degeneration and vascularization of tumors.
- Since it is essential to survival, angiogenesis is facilitated by redundant, compensatory mechanisms and is probably activated by multiple pathways.
- In a series of animal studies, angiostatic monotherapy resulted in upregulation of proangiogenic factors, probably by compensatory mechanisms. However, angiostatic combination therapy with compounds targeting different parts of the angiogenic process resulted in significantly less upregulation.
- These studies demonstrated that angiostatic combination therapy has synergistic angiostatic and antineovascular effects and required much lower doses than monotherapy to demonstrate angiostatic effect.
- Combination therapy with three different anti-vascular endothelial growth factor (anti-VEGF) compounds did not result in improved angiostatic activity compared with anti-VEGF monotherapy.
- Combination therapy obliterated tumor vasculature and prolonged survival, while the effects of monotherapy were similar to the vehicle control.
- When applied to all neovascularization models (developmental, pathological and tumor), angiostatic combination therapy did not affect normal vasculature.
- These studies point to the utility and effectiveness of combination therapy in the clinic. Even though anti-VEGF therapy is currently the only available angiostatic therapy for ocular CNV (making combination therapy with different angiostatic therapies impossible) the studies reported in Dorrell and colleagues support the concept of combination therapy using agents with different mechanisms of action.
References
Papers of special note have been highlighted as:
• of interest
• Interesting overview regarding the many physiological tasks of vascular endothelial growth factor (VEGF).
• Shows for the first time that photodynamic therapy results in enhanced VEGF-expression and thus confirming the theoretical concept of combination approaches as being most suitable.
• Shows for the first time that the concept of eradicating the choroidal neovascularisation by means of a physicochemical approach in combination with the inhibition of inflammation and VEGF works in a clinical setting.

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