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Benefits and Risks of Inhibiting Vascular Endothelial Growth Factor (VEGF) at the Ocular Level: From Bench to Bedside

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Intravitreal anti-vascular endothelial growth factor (VEGF) therapies (ranibizumab and bevacizumab) have revolutionized the treatment of many ocular diseases including age-related macular degeneration (AMD),[1,2] diabetic, macular edema (DME),[3] retinal vein occlusion (RVO),[4,5] and even retinopathy of prematurity.[6] Yet, their use is under continuous scrutiny in regard to potential localized and systemic adverse events (AEs), the risk of cardiovascular (CV) events in particular.[7-13] Furthermore, while ranibizumab has been approved for intraocular administration, bevacizumab (indicated for the systemic treatment of various cancers) is often used interchangeably. Off-label use of a drug to treat a condition for which another agent has been approved creates medical and legal controversies. The lack of a large randomized clinical trial (RCT) that is powered to clearly differentiate the safety profiles of ranibizumab and bevacizumab largely contributes to ongoing safety related debates. Such a trial is unlikely to be conducted due to various constraints that are mainly related to the requirement of a relatively large sample size (>20 000 patients) that would provide power to detect statistically significant differences between the agents. In the absence of such a trial, signals from efficacy-driven RCTs, as well as population based studies, meta-analyses and observational studies need to be considered. The objective of this issue of *Ophthalmology Scientific Update* is to provide ophthalmologists with an in-depth overview and expert opinion concerning current evidence in regard to the safety of intravitreal anti-VEGF therapies as it pertains to daily clinical practice and specific patient profiles.

The Critical Role of VEGF Signaling in Angiogenesis and CV Homeostasis

The role of VEGF in embryonic development

The VEGF signaling pathway, as demonstrated by genetic ablation experiments in mice, is the most critical driver of vessel formation.[14-16] It induces emergence, differentiation, proliferation, and migration of endothelial cells, which leads to the development of the CV system through both vasculogenesis (*de novo* tube formation) and angiogenesis (sprouting of new tubes from pre-existing tubes).[16] Angiogenesis occurs in several well-characterized stages.[17] The first stage involves biological signals that activate receptors present on endothelial cells in already existing blood vessels. The activated endothelial cells release proteases that degrade the basement membrane to allow endothelial cells to escape from the parent vessel walls. The endothelial cells then proliferate into the surrounding matrix and sprout toward the source of the angiogenic stimulus. These sprouts form loops that become a vessel lumen.

Vessel formation is a highly dynamic process that involves intense remodeling.[18] Recent evidence has shown that specific cells situated at the extremities of capillary sprouts, named tip cells, control branching of blood vessels and that this branching is controlled by VEGF gradients.[19,20]

Expression of the gene for VEGF, stimulated under hypoxic conditions, is regulated through transcriptional and post-transcriptional mechanisms.[21,22] VEGF acts through 2 high-affinity tyrosine kinase receptors (VEGFR-1 and VEGFR-2) that are expressed on almost all organ tissues and are upregulated during angiogenesis.[23-25] While VEGFR-2 is thought to mediate most of the angiogenic functions attributed to VEGF, the role of VEGFR-1 signaling is less clear. Some consider it to be a decoy receptor.[26]

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VEGF signaling pathway in CV and ocular homeostasis

Experiments in animal models also demonstrate the critical role of VEGF and VEGF signaling in the homeostasis of the adult vascular system.[24,27] VEGF is essential for endothelial survival, CV repair, and regeneration. Damage to the endothelium is believed to be one of the most important early events in the development of atherothrombosis, such as myocardial infarction (MI) and strokes. VEGF works through an intricate pathway to stimulate the production of nitric oxide (NO), which has been well described as being the most important mediator of vascular health. Through promoting endothelial health, VEGF protects against vascular injury, and exerts important anti-inflammatory and anti-thrombotic properties. Hence, blockade of VEGF, particularly systemically, can elicit potential deleterious effects on the CV system, and in fact on multiple organ systems given the essential role of vascular perfusion.

In 65% of mutant mice, genetic deletion of VEGF in the endothelial lineage led to progressive endothelial degeneration, microhemorrhages, intravascular thrombosis and sudden death by 25 weeks of age. Furthermore, the deletion of endothelial VEGF did not result in detectable changes in the levels of circulating VEGF or in levels of total VEGF mRNA in all of the organs examined. This indicates that paracrine VEGF could not compensate for the deficiency in endothelial VEGF.[24] Izumiya et al[27] showed that VEGF blockade promotes rapid progression from compensatory cardiac hypertrophy to failure in pressure-overloaded murine hearts.

VEGF and pathological angiogenesis

Conversely, the same VEGF signaling that is critical in mediating angiogenesis and implicated in different developmental processes and CV homeostasis is also a potent stimulator of pathological angiogenesis.[28,29] Numerous experiments have unequivocally demonstrated that, in adults, VEGF is required for pathological growth of vessels in many conditions including inflammation,[30] arthritis,[31] retinopathies,[32] and malignancies.[28] Thus, anti-angiogenesis agents that target the VEGF signaling pathway have become an important part of standard therapy in multiple angiogenesis-driven conditions, including cancer and retinopathies. Anti-VEGF based therapies that work locally within the eye and exhibit physical characteristics consistent with minimal systemic exposure present an ideal solution for treating the debilitating symptoms and restoring vision in AMD patients.

Potential CV Effects of Inhibiting VEGF

It is well documented that vascular stress and/or injury cause upregulation of VEGF.[25] Interactions of VEGF with VEGFR-2 on endothelial cells leads to production of NO and prostaglandin 1, an increase in endothelial-cell permeability, proliferation, migration, and survival. These VEGF-dependent effects are essential for interactions between endothelial cells and platelets, vasodilation, and prevention of adherence of blood cells to the endothelial cell lining. Inhibition of VEGF signaling may therefore impair vascular homeostasis and physiological response to stress, which in turn might lead to compromised wound healing, hypertension, arterial thrombosis, cardiac dysfunction, proteinuria, and renal adverse effects (Table 1).[25]

The Unique Intersection of AMD and CV Disease: Cause or Effect?

Numerous studies support the notion that AMD is associated with underlying systemic vascular disease, including ischemic and hemorrhagic incident stroke[33,34] and myocardial infarction (MI).[35] Furthermore, many of the traditional risk factors for CV and cerebrovascular diseases (ie, diabetes, hypertension, smoking, unhealthy diet, sedentary lifestyle) are also associated with AMD.[36,37] Hogg et al[38] suggested that CV disease plays an etiological role in the development of choroidal neovascularization (CNV) in a proportion of older adults.

Intravitreal Anti-VEGF Therapies and the Risk of CV Disease

The systemic safety of intravitreally administered anti-VEGF therapies has never been fully established. Intraocular administration should, however, result in much lower systemic AEs com-

Table 1: Possible mechanism of adverse effects related to systemic VEGF inhibition in cancer patients

Hypertension
- Decrease in nitroxide and prostaglandin I_2 production leading to inhibition of vasodilatation
- Decrease in arteriole and capillary density (rarefaction)

Arterial thrombosis
- Endothelial cell apoptosis
- Disturbance of platelet-endothelial cell homeostasis; platelet aggregation
- Exposure of extracellular matrix to blood cells

Cardiomyopathy
- Increase in peripheral vascular resistance
- Inhibition of VEGF-dependent cardiomyocyte growth in response to ischemia or blood pressure elevation
- Ischemic changes in coronary arterioles

Preteinuria and renal adverse effects
- Disturbance of VEGF-dependent function and interaction between endothelial cells and podocytes in the filtration barrier of glomeruli
- Thrombotic microangiopathy
- Endothelial cell damage

Wound healing issues
- Impaired neovascularization
- Disturbance of platelet-endothelial cell interaction
- Reduction in the VEGF-induced tissue factor on endothelial cell results in compromised coagulation cascade and platelet activation

Bowel perforation
- Ischemic changes in intestinal walls
- Impaired wound healing

VEGF = vascular endothelial growth factor

pared to the systemic use in oncology. There are also many unknowns in regard to the pharmacokinetics of anti-VEGF agents in the eye.

Molecular structure, pharmacology and pharmacokinetics of anti-VEGF agents

It has been suggested that bevacizumab (full-length antibody) has a longer half-life in the eye than ranibizumab (antibody fragment). The crystallizable fragment (Fc) of bevacizumab also facilitates transport of the molecule from the eye into the circulation, creating a theoretically higher risk of systemic AEs compared to ranibizumab.[39]

In rabbits, according to Bakri et al,[40] the vitreous half-life is 2.88 days for ranibizumab and 4.32 for bevacizumab. Furthermore, small amounts of intravitreal bevacizumab, but not ranibizumab, have been detected in the fellow uninjected eye and in serum.[40] Radioactivity assays in animals have also indicated that both agents penetrate all retinal layers including the retinal pigment epithelium (RPE).[41,42] Radioactivity was also present in serum for up to 7 days following intravitreal injection with radioactive bevacizumab.[41] Recently, studies by Barros-Pereira et al[43] and Carneiro et al[44] revealed significant reductions in VEGF plasma levels during the first 28 days after intravitreal bevacizumab injection in AMD patients (*P*=0.00006 and 0.0002, respectively). In contrast, intravitreal ranibizumab was not associated with significant reductions in systemic VEGF levels.

Evidence from clinical trials and population-based studies

In the large RCTs of ranibizumab, adverse systemic events were rare.[10] Pooled data from 3 ranibizumab trials (MARINA, ANCHOR, and FOCUS) revealed atherothrombotic event (ATE) rates of 4.8%, 4.3% and 4.4% in the ranibizumab 0.5 mg and 0.3 mg, and the control group, respectively. The difference in rates between ranibizumab groups and control was not statistically significant.[45] There has, however, been some indication of increased risk of stroke with ranibizumab 0.5 mg.[45,46] Pooled analysis of 3 ranibizumab trials revealed higher, but not significantly, rates of stroke in the 0.5-mg group (2.7%) at 2 years compared to control group (1.1%; *P*=0.15).[45] An interim analysis of the SAILOR trial involving approximately 2 400 patients suggested that there was an increased risk of stroke in patients receiving the higher dose of ranibizumab (1.2% for the 0.5-mg group vs. 0.3% for the 0.3-mg group; *P*=0.02). At 1 year, the stroke incidence remained higher in the 0.5 mg group but the difference between the groups was not statistically significant. Patients with a prior history of stroke appeared to be at higher risk, but there was no increased risk of MI or vascular death.[46]

A literature review of 22 clinical studies (N = 12 699) involving intravitreal bevacizumab pointed to increased blood pressure (0.46% of patients), cerebrovascular events (0.21%), and MI (0.19%) as the most common bevacizumab-related systemic AEs.[47] Yet, the number of patients included in the individual bevacizumab studies remains too small to draw any firm conclusions regarding its CV safety, especially in relation to ranibizumab.

Two recent studies added to the growing debate regarding the use of ranibizumab versus bevacizumab in patients with AMD. In the Comparison of Age-Related Macular Degeneration Treatments Trial (CATT) the rates of atherothrombotic and venothrombotic events and mortality did not significantly differ between ranibizumab and bevacizumab.[48] However, more patients treated with bevacizumab experienced serious systemic AEs – primarily hospitalizations – compared to ranibizumab-treated patients (24.1% vs. 19.0%; *P*=0.04; Table 2). This risk of serious systemic events remained 29% higher after adjustment for demographic features and coexisting illnesses at baseline (*P*=0.04). Although no specific organ system consistently accounted for the difference in AEs, differences in rates for infections and gastrointestinal disorders were the highest. It should, however, be reiterated that the CATT trial was not primarily designed or sufficiently powered to conclusively establish differences in AEs between the 2 compounds.

In a recent retrospective population-based study that included almost 147 000 Medicare beneficiaries with a claim for AMD, a significantly lower risk of stroke was noted in patients treated with ranibizumab compared to bevacizumab (hazard ratio [HR] 0.81; 99% confidence interval [CI] 0.68-0.98).[49] In addition, the secondary analysis that included only patients who received either bevacizumab (N=21 815) or ranibizumab (N=19 026) as first-line therapy, reported significantly lower HRs of mortality (0.86; 95% CI 0.75-0.98) and stroke (0.78; 95% CI 0.64-0.96) in patients treated with ranibizumab compared to bevacizumab (Figure 1). Further analysis conducted by Gower et al[50] indicated an 11% higher risk in all-cause mortality (HR 1.11; 99% CI 1.01-1.23) and a 57% higher risk of hemorrhagic cerebrovascular accident (HR 1.57; 99% CI 1.04-2.37) in individuals

Table 2: CATT: Selected adverse events within 1 year after enrolment

	Ranibizumab Monthly	Bevacizumab Monthly	Ranibizumab PRN	Bevacizumab PRN
N (safety analysis set)	301	286	298	300
Deaths from any cause	4 (1.3%)	4 (1.4%)	5 (1.7%)	11 (3.7%)
Arteriothrombotic events	7 (2.3%)	7 (2.3%)	6 (2.0%)	8 (2.7%)
Nonfatal MI	2 (0.7%)	2 (0.7%)	3 (1.0%)	1 (0.3%)
Nonfatal stroke	3 (1.0%)	3 (1.0%)	1 (0.3%)	2 (0.7%)
Death from vascular causes	2 (0.7%)	2 (0.7%)	2 (0.7%)	5 (1.7%)
Venous thrombotic events	0	4 (1.4%)	2 (0.7%)	1 (0.3%)
≥ Systemic SAEs[a]	53 (17.6%)	64 (22.4%)	61 (20.5%)	77 (25.7%)

[a] 24.1% vs 19.0%; risk ratio 1.29 (95% confidence interval 1.01–1.66, *P* = 0.04); mainly hospitalizations and gastrointestinal disorders

CATT = Comparison of the Age-related Macular Degeneration Treatment Trial; PRN = as needed; MI = myocardial infarction; SAEs = serious adverse events

Adapted from CATT Research Group. *N Engl J Med.* 2011;364(20):1897-908.

(N=77 886; 46% ranibizumab) treated with bevacizumab compared to ranibizumab.

Intravitreal Anti-VEGF Therapies and the Risk of Ocular AEs

Endophthalmitis and serious intraocular inflammation

In ranibizumab clinical trials, ocular AEs were rare. Per injection rates for presumed endophthalmitis and serious intraocular inflammation were 0.05% and 0.03 %, respectively.[51] The reported rates of inflammation for bevacizumab vary from 0.1% to 0.8%.[52-55] In the CATT trial, ocular AEs occurred in <1% of patients.[48] There were 6 cases of endophthalmitis (2 among monthly ranibizumab users and 4 cases among monthly bevacizumab users [*P*=0.45]), as well as 1 case of pseudo-endophthalmitis in the monthly ranibizumab group. However, both drugs in this trial were supplied in glass vials.[56] In daily practice, ranibizumab is supplied in unit-dose packaging and off-label bevacizumab requires aliquoting and storing in plastic syringes. This practice of aliquoting intraocular doses from a larger single-use vial raises some concerns about additional risk of contamination during the aliquoting process, as underlined by reports of case clusters of bevacizumab-associated ocular AEs, including intraocular inflammation.

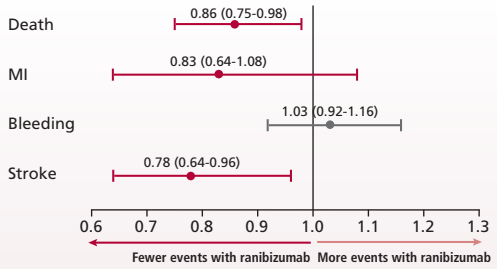
In Canada, there were several reports in October 2008 of a syndrome labeled as toxic anterior segment syndrome (TASS)/sterile endophthalmitis after intravitreal injection with a specific lot number of bevacizumab.[57] As a result, it was recommended to suspend the intravitreal use of bevacizumab from this lot number. In December 2008, Health Canada requested that Roche issue a letter to healthcare professionals indicating that bevacizumab is not authorized for use in the ophthalmology setting.[58]

During the summer of 2011, clusters of severe cases of *Streptococcus endophthalmitis*, some of which resulted in blindness and brain injuries, were reported in Florida and Tennessee.[59] In Florida, cases were associated with the use of bevacizumab and linked to a compounding pharmacy in Hollywood, Florida. The pharmacy had repackaged bevacizumab from sterile 100-mg/4-mL, single-use, preservative-free vials into individual 1-mL syringes. Such practice, performed without proper aseptic considerations, can lead to bacterial contamination. Thus, the Food and Drug Administration (FDA) issued a letter alerting healthcare professionals of infection risk from using repackaged bevacizumab for intravitreal injections.[60] Another letter was requested by Health Canada in December 2011, in which Roche reiterated that bevacizumab is not formulated for intravitreal use.[61]

Increase in intraocular pressure

The short-term transient increase in intraocular pressure (IOP) immediately after intravitreal anti-VEGF therapy is a well-described phenomenon.[62] Recent reports also suggest that sustained IOP after intravitreal anti-VEGF treatment is also possible.[63-65] Tsang et al[65] identified 25 previously normotensive eyes that developed sustained ocular hypertension after anti-VEGF therapy for neovascular AMD. The majority of the intravitreal injections were ranibizumab (453 of 499, 90.8%), and 11 of the 25 eyes had been treated with ranibizumab alone. However, from the available data it was impossible to determine whether ranibizumab is more likely to lead to sustained ocular hypertension than bevacizumab.

Figure 1: Adjusted hazard ratios of adverse events at 1 year in patients receiving ranibizumab compared with bevacizumab as first-line therapy



N= 40 841 (21 815 bevacizumab and 19 026 ranibizumab patients)
Curtis LH, et al. *Arch Ophthalmol.* 2010;128(10):1273-1279.

An Overview of Regulatory Agency Positions

The concern over AEs is amplified when a drug is used off-label due to possible significant underreporting of such events. Due to concerns over the risk of adverse effects, the Royal College of Ophthalmology in the United Kingdom has recommended against the use of intravitreal bevacizumab for the treatment of wet AMD.[66]

The recent FDA Guidance for Industry regarding the evaluation of CV risk in new antidiabetic therapies to treat type 2 diabetes mellitus, state that it is important to recognize that signals of CV harm cannot be interpreted conclusively based on smaller efficacy-driven trials.[67] If feasible, these small signals should be evaluated in large prospective trials that are adequately powered. Furthermore, in the absence of large trials, the FDA requires careful post-marketing surveillance and risk stratification for all drugs with an HR over 1.3. It is also important to keep in mind that small signals from small trials have yielded significant harm in large meta-analyses and resulted in drugs being withdrawn from the market (eg, rosiglitazone, rofecoxib).

Conclusion

From a scientific standpoint, the available evidence is consistent with differences in the pharmacology of ranibizumab and bevacizumab. There is evidence now in humans that intravitreal therapy with bevacizumab in AMD is associated with a reduction in systemic VEGF levels, which has so far not been observed with ranibizumab. This evidence may provide credible biological plausibility of differential CV toxicity, and is supported by several population-based comparative approaches. It is also important to appreciate that the current Canadian bevacizumab product

monograph has been updated to highlight the systemic AEs of intravitreal therapy noted above.[68]

However, a satisfactory conclusion surrounding safety debates regarding intravitreal anti-VEGF therapies remains elusive despite the availability of 1-year CATT results. In the absence of more convincing clinical trial data, population-based studies, meta-analyses and pooled analyses from small scale clinical trials provide the best safety related evidence regarding the use of anti-VEGF agents in AMD patients. As it is unlikely that any of the current clinical trials will provide a definite answer about systemic safety, there is an increased need for surveillance programs and safety databases with patients on the different anti-VEGF therapies for various ocular conditions. Due to advanced age and various comorbid conditions, virtually all AMD patients are at risk of CV and other AEs associated with VEGF inhibition and/or improper preparation and administration procedures.

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