Indirect blockade of VEGF: the potential for eye disease therapy

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Summary

The process of neovascularization is stimulated by proangiogenic growth factors, particularly vascular endothelial growth factor (VEGF), and the use of intravitreally injected anti-VEGF agents is currently the gold standard of care in the treatment of neovascular retinal pathologies. However, caution is needed, as anti-VEGF agents display a variety of limitations and adverse side effects. In addition, there is evidence that VEGF is a neuroprotective factor supporting retinal neurons independently of its actions on vessels, thus direct VEGF blockade may interfere with neuronal survival. Therefore, additional therapies are needed to reduce these potential adverse effects of VEGF blockade. A number of new therapeutic approaches are being tested for their ability to indirectly target the VEGF system reducing retinal VEGF levels or the efficiency of VEGF signaling. Here, we briefly report some experimental observations on substances that may indirectly inhibit the deleterious effects of VEGF in eye disease.

Keywords

Retinopathies

Somatostatin

Nutraceuticals

Beta adrenergic system

Glucocorticoids

Renin angiotensin system

Unfolded protein response

Direct vascular endothelial growth factor (VEGF) blockade through intravitreal injections of anti-VEGF drugs has high success rates in the treatment of different neovascular retinal pathologies, including diabetic retinopathy (DR), diabetic macular edema (DME), retinopathy of prematurity (ROP) and age-related macular degeneration (AMD). Although DR, DME, ROP and AMD are different diseases, with different medical needs and different responses to direct anti-VEGF blockade, this approach, in general, may cause ocular or systemic side effects and patients may be refractory [1,2]. In addition, the use of direct VEGF blockade in developing individuals, for instance for the treatment of ROP, might interfere with normal angiogenesis or induce persistent changes in neural retinal structures [3]. Indeed, there are numerous reports of neuroprotective actions of VEGF [4], suggesting that VEGF release in early phases of retinal disease, for instance in early DR, is unlikely to be aimed at pro-angiogenic effects but, rather, is related to a protective strategy of the retina. Therefore, it is necessary to determine which of the two components (the effects on neovascularization and those on neuronal survival) may dominate the response of the retina to a potential treatment: the optimal choice would be a drug that only targets the "bad" VEGF, which causes neovascular damage, while preserving VEGF levels for neuronal survival. Basic research on animal models is investigating possible strategies based on indirect VEGF blockade to offer new perspectives to the patients. In addition, the identification of substances that could be administered with eye drops or, even better, as nutraceuticals would represent a further advantage.

Indirect VEGF blockade may be obtained by interfering with the VEGF biosynthetic pathways that are activated in conditions of retinal stress such as those in DR, ROP or AMD, but increased VEGF expression may be also prevented acting upstream, that is protecting the retina from the metabolic stress that eventually would cause VEGF upregulation. This might be obtained by reducing oxidative stress, inflammation, or neuronal damage.

Regulation of VEGF expression

Hypoxia plays a major role in ocular diseases characterized by neovascularization, and the hypoxia inducible factor 1 (HIF-1) acts as a major transcriptional regulator of VEGF [5]. In addition to HIF-1, other transcription factors, such as signal transducer and activator of transcription 3 (STAT3), also regulate VEGF expression [6]. Drugs interfering with these or other VEGF regulators may indirectly inhibit the deleterious effects of VEGF in eye disease. A wide variety of substances has been investigated in this respect using both *in vitro* and *in vivo* models, and only an extensive review could account for all these studies. In this editorial, we will present briefly the substances whose effects on VEGF expression are supported by consolidated experimental evidence.

Somatostatin. Somatostatin acting at its sst_2 receptor reduces both retinal neovascularization and VEGF expression in a mouse model of ROP [7,8]. Results from this model and from mouse *ex vivo* retinal explants show that activation of sst_2 prevents hypoxia-induced VEGF upregulation acting directly on VEGF-expressing endothelial cells, thus implying that sst_2 expressed by retinal vessels receives an angioinhibitory action from sst_2 agonists [9,10]. Experiments in *ex vivo* mouse retinal explants cultured in hypoxia have clarified that sst_2 stimulation leads to the activation of Src homology region 2 domain-containing phosphatase 1 (SHP-1), which reduces hypoxic levels of pSTAT3 and HIF-1α, thus preventing hypoxia-induced VEGF up-regulation [10]. Clinical studies indicated that somatostatin levels in the vitreous of patients with DR are lower than in non-diabetic subjects [11], suggesting that a somatostatin deficit may contribute to the development of DR. The assessment of the clinical efficacy of somatostatin analogs in DR has produced conflicting results [12], but subcutaneous administrations of octreotide (a long-lasting sst_2 agonist) in humans have been reported to retard the progression of DR and to delay the time to laser surgery [13]. Presently, the ongoing EUROCONDOR clinical trial is evaluating the effects of somatostatin administered as eye drops in the treatment of early stages of DR [14]. Interestingly, somatostatin eye drops prevent retinal neurodegeneration in a rat model of DR [15].

Plant extracts. Extracts from a variety of plants used in traditional medicine are capable of inhibiting neovascularization in animal models of eye disease acting negatively on VEGF [2]. For instance, bilberries (*Vaccinium myrtillus*) and curcumin have been reported to reduce VEGF expression in rat models of DR [16,17]. Among natural substances with potential nutraceutical value, we found that acetyl-11-keto-β-boswellic acid (AKBA), an active principle derived from the plant *Boswellia serrata*, efficiently inhibits pathologic neovascularization in a mouse model of ROP. Similar to somatostatin, AKBA is likely to reduce VEGF expression affecting the SHP-1/STAT3/VEGF axis [18].

β-adrenoreceptors (β-ARs). Studies on the retinal β-adrenergic system have demonstrated that β-AR blockade results in strong inhibition of vascular changes in *in vivo* models of proliferative retinal diseases. In particular, systemic administrations of the β1-/β2-AR blocker propranolol effectively inhibit both the increase of retinal VEGF expression caused by hypoxia and the consequent neovascular response. Clinical trials prompted by these observations have shown that oral administrations of propranolol to preterm infants with ROP result in positive outcomes in terms of efficacy, although safety problems are also present. Recently, experimental studies in a mouse model of ROP showed that topical propranolol administrations as eye drops are have the same effects on VEGF and neovascularization as the systemic administration, opening new perspectives for possible use in humans [4].

Glucocorticoids. Glucocorticoids exert indirect effects on VEGF by blocking up-stream pro-inflammatory proteins, but they also decrease VEGF expression *in vitro* [19]. The steroids triamcinolone, dexamethasone and fluocinolone, are clinically used in the treatment of DME [20]. Advantages of glucocorticoids are that they may be administered less frequently than anti-VEGF agents and that they can be used in combination with other therapies. However, the impact and the intensity of their effects have not been shown in the clinical routine. In addition, they are short-lived and their effects are transitory. Systemic glucocorticoid administration may lead to systemic side

effects, therefore only intraocular delivery is recommended. These limitations led to the development of methods for sustained intravitreal steroid release. For instance, a Phase III clinical trial has demonstrated that dexamethasone intravitreal implants may provide retinal drug delivery for about 6 months and recently have been approved for use in the treatment of DME [21].

Renin-angiotensin system (RAS). RAS plays an important role in the regulation of blood pressure and fluid balance. In DR, the retinal RAS is upregulated, with increased levels of renin, angiotensin converting enzymes (ACE) and angiotensin receptors (ATR). Inhibitors of ACE, ATR or aldosterone synthase reduce retinal neovascularization and VEGF expression in rodent models of ROP and DR. Some benefit of RAS blockade in DR has been reported in clinical trials [22].

Unfolded protein response (UPR). UPR is activated in vascular endothelial cells upon accumulation of unfolded or misfolded proteins in the endoplasmic reticulum (ER), a condition known as ER stress, when cells are exposed to hypoxia/ischemia, inflammation, or oxidative stress. Molecular chaperones, which normally function to facilitate protein folding in the ER, regulate angiogenic factor production and are involved in the UPR. Targeting such molecules inhibits VEGF expression [23].

Protection of the retina from metabolic stress

We have observed in *ex vivo* mouse retinal explants that an ischemic condition, typical of different retinal pathologies, provokes increased VEGF expression and release, and that these effects are inhibited by neuroprotection provided by neuropeptides [24,25]. Therefore, the idea is that increasing VEGF expression and release is the first response of the retina to a metabolic stress and that protecting retinal cells from damage may effectively result in VEGF reduction. Indeed, neurodegeneration is recognized as an early event in the pathogenesis of DR and strategies based on neuroprotection may constitute a new therapeutic approach for DR [26].

As described above, the neuropeptide somatostatin inhibits VEGF expression acting on the SHP-1/STAT3/VEGF axis [10], but the possibility exists that this effect is also due to somatostatin neuroprotective actions [24,25]. Similarly, AKBA limits VEGF production affecting SHP-1 and STAT3 [18], but this action may be due, at least in part, to the anti-inflammatory properties of AKBA, demonstrated *in vitro*, *in vivo* and in clinical trials [27], which would reduce the level of metabolic stress in the retina. In general, plants used in traditional medicine with proven neuroprotective, anticancer, immunomodulatory and antioxidant activities have been found to reduce VEGF in models of eye disease [2]. The same concept may apply to glucocorticoids, whose anti-VEGF effects may be due to their well-established anti-inflammatory and neuroprotective properties demonstrated in humans [19], and to retinal RAS, whose blockade may reduce inflammation and oxidative stress [22].

Conclusion

Indirect VEGF inhibition would represent a significant step forward in the treatment of eye disease in comparison to approaches based on direct VEGF blockade. However, there are potential side-effects of drugs targeting mediators participating to the modulation of VEGF expression but that may also be involved in a variety of other intracellular pathways. To avoid these problems, VEGF expression should be prevented mainly upstream (by reducing inflammation, oxidative stress or neuronal apoptosis), and the best drugs would be those that do not need delivery through intraocular injections. Some of the substances described above may meet these criteria. For instance, somatostatin (or its agonist octreotide) is neuroprotective and has a specific action limiting VEGF expression; AKBA is a nutraceutical (that can be delivered with the diet) and has both general anti-inflammatory and specific anti-VEGF effects; the β1-/β2-AR blocker propranolol inhibits VEGF and can be delivered through topical application with no documented systemic side effects in humans. However, at present, a major concernregarding several of these promising alternatives to

direct anti-VEGF agents is that their effectiveness has not been fully tested in the clinical routine, and possible side effects have not be evidenced so far. On the other hand, these substances, different from direct anti-VEGF agents binding all vitreous VEGF, are good candidates to target the "bad" VEGF, which causes neovascular damage, while preserving basal VEGF levels. For instance, propranolol reduces VEGF overproduction in hypoxic retinas with a trend toward recovering control levels but it does not seem to affect VEGF levels in normoxic retinas, suggesting different patterns of regulation of VEGF expression in normoxic and hypoxic conditions [4]. A stimulating perspective is the idea of using some of these substances together to obtain a compound with anti-inflammatory, anti-oxidative and neuroprotective actions added to the ability of interfering with the VEGF biosynthetic pathway.

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