Novel approach to antiangiogenic factors in age-related macular degeneration therapy

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Abstract

Age-related macular degeneration (AMD) is a leading cause of irreversible vision loss among the population above 85 worldwide. There are two main types of AMD: neovascular and dry AMD. Neovascular AMD leads to macular changes resulting from abnormal choroidal neovascularization. Untreated neovascular AMD leads to scar formation and irreversible sight deterioration. Dry AMD in consequence leads to atrophic changes of the macula.

The last decades brought a breakthrough in the therapy of neovascular age-related macular degeneration by introduction of, firstly, photodynamic therapy and, later, anti-VEGF agents administered intravitreally in order to stop neangiogenesis. However, the treatment of dry AMD is still challenging. Among the directions in dry AMD treatment, the most promising are complement cascade inhibitors and complement cascade targeted gene therapy.

In the article we outline the main directions in up-to-date experimental and practical approaches to wet and dry AMD therapy with the emphasis on antiangiogenic factors and gene therapy focused on the inhibition of pathological angiogenesis.

Key words: age-related macular degeneration, axitinib, AMD, eculizumab, gene therapy, brolucizumab, abicipar pegol, faricimab, lampalizumab, pegcetacoplan.


Introduction

Age-related macular degeneration (AMD) is a disorder of the macula – the central region of the retina. It is one of the leading causes of irreversible visual impairment. It affects around 3.6 million people worldwide [1]. It is estimated to affect 13.1% of the population older than 85 years [2].

There are several approaches to AMD classification, including the Three Continent AMD severity scale, which distinguishes 5 stages of the disease: no AMD, mild early, moderate early, severe, and late [2]. Another classification differentiates wet AMD and dry AMD. Wet AMD (or neovascular AMD; nAMD) is an effect of choroidal neovascularization (CNV). In type 1 the changes are located below the retinal pigment epithelium (RPE). In type 2 (“classical choroidal neovascularization”) they involve pathological blood vessel formation within the interior layers of the retina. In type 3 the anastomoses between neovascularization and retinal circulations are present. The neovascularization leads to the presence of fluid or hemorrhage in the retinal tissues, retinal pigment epithelial detachments and hard exudates. The irreversible stage of neovascular AMD is subretinal fibrous scar tissue formation [3, 4].

Dry AMD (atrophic AMD) involves outer retinal thinning. The early stage is characterized by accumulation of lipoid extracellular deposits of metabolic products called drusen [5]. Secondary degeneration of the retinal pigment epithelium proceeds and then the degeneration of the choriocapillaris takes place. It manifests as geographic atrophy [3, 4].

Pathological neovascularisation formation is related to imbalance between reactive oxygen species (ROS) and antioxidants. The imbalance leads to oxidative stress and, in consequence, to formation of oxidative stress mediators (e.g. vascular endothelial growth factor – VEGF) by Muller cells and retinal microglia [6, 7].

Vascular endothelial growth factor is an agent produced by hypoxic tissues in order to stimulate vessel growth, as it promotes neovascularization (NV), regulates vascular permeability and, in consequence, triggers the formation of CNV in nAMD [3]. Due to the introduction of anti-VEGF treatment, the prevalence of blindness due to AMD decreased by almost 30% from 1990 to 2020 [1]. Anti-VEGF therapy is effective in neovascular AMD, where the patho-
logical angiogenesis is suppressed by VEGF inhibitors. Nowadays, non-exudative AMD has become a challenge for inventing new therapies.

**Photodynamic therapy (verteporfin)**

Photodynamic therapy (PDT) with verteporfin is a well-known approach to treat CNV in AMD. Apart from the most common use, which is nAMD, verteporfin therapy is applied in other conditions associated with choroidal pathologies, such as polypoidal choroidal vasculopathy, central serous chorioretinopathy, and choroidal hemangioma. Its effect is exerted by angioocclusive action of light energy with the presence of a photosensitizer [8-10].

Verteporfin (VP) is a photosensitizer used commonly in ophthalmology. Apart from it, there have been proposed other substances varying in the level of toxicity and affinity to the treated tissues, such as a hematoporphyrin derivative, peptides, and monoclonal antibodies [11].

Photodynamic therapy has also been used in non-ocular conditions such as psoriasis, actinic keratosis and malignancies such as esophageal, lung and skin cancers. The use of verteporfin has been proven successful in choroidal metastases of breast, thyroid, lung and pancreas cancer [12].

Nowadays, the use of PDT in AMD has become less common due to the advances in anti-VEGF treatment.

**Advances in anti-VEGF therapy**

Angiogenesis is regulated in multiple mechanisms. VEGFs and their receptors (vascular endothelial growth factor receptors – VEGFRs), as well as placental growth factor (PIGF), angiopoietin 2 (Ang-2), platelet-derived growth factor-B (PDGF-B), vascular endothelial-protein tyrosine phosphatase (VE-PTP) and stromal-derived growth factor (SDF-1) promote angiogenesis secondary to elevation of hypoxia-inducible factor-1 (HIF-1) level upregulation. The subtypes of VEGFRs that are most important for AMD pathogenesis are VEGFR1 and VEGFR2. VEGFs’ main subtypes are VEGF-A and VEGF-B. VEGF-A binds with both VEGFR-1 and VEGFR-2, whereas VEGF-B (as well as PIGF) binds selectively with VEGFR-1 [7].

VEGF leads to vascular leakage and neovascularization [7, 13, 14]. Pathological angiogenesis in eye tissues is induced by VEGF formation in tissues such as retinal pigment epithelium and non-pigmented ciliary epithelium [15].

Anti-VEGF agents are used for treating retinal neovascularization, such as in AMD, diabetic retinopathy and secondary to central retinal vein occlusion. The route of administration is by intra-vitreal injection, which limits the systemic side-effects [13].

Pegaptanib sodium (Macugen; Eyetech Pharmaceuticals/Pfizer) was the first anti-VEGF agent in use for AMD. Pegaptanib binds large-sized VEGF isoforms, such as VEGF165, which limits its effectiveness. The anti-VEGFs clinically approved and currently in use are: bevacizumab (Avastin; Roche), ranibizumab (Lucentis; Novartis), afibercept (Eylea; Bayer) and brolucizumab (Beovu; Novartis). VEGF-A isoforms of VEGF are bound by bevacizumab and ranibizumab. Bevacizumab is a recombinant humanized monoclonal antibody of VEGF, whose structure contains two light and two heavy chains. It finds its main use in oncology. Ranibizumab is a lightweight humanized monoclonal antibody fragment targeted against VEGF-A isoforms.

Aflibercept is a recombinant protein binding to VEGF-A and VEGF-B isoforms as well as PIGF. It may be administered in an every-2-month regimen. It has been known safe and efficient since 2012 [3, 16].

Brolucizumab is the newest agent approved by the FDA (United States Food and Drug Administration) in 2019. It is a single-chain anti-VEGF-A antibody fragment. Brolucizumab was proven non-inferior to aflibercept in terms of visual function and similar to aflibercept in terms of overall safety in the HAWK and HARRIER trials [17] (Fig. 1, Table 1 [18-29]).

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**Fig. 1.** The targets of anti-VEGF agents and the effect on vascular leakage and angiogenesis
New anti-VEGF agents in neovascular AMD

There are current studies developing novel anti-VEGF treatment for wet AMD. One such agent is abicipar pegol. It is the first non-monoclonal antibody protein in the anti-VEGF group.

Abicipar pegol has a low molecular weight and no immunoglobulin component. Its structure is based on a repeated sequence of proteins called DARPins: designed ankyrin repeat proteins. DARPins are designed and engineered to bind specifically to a ligand, in this case to VEGF.

In phase 3 trials, CEDAR (NCT02462928) and SEQUOIA (NCT02462486), comparing the effect of abicipar pegol to ranibizumab it was found that it maintained stable vision [25, 26]. Other trials showed longer half-life of abicipar pegol than of aflibercept and ranibizumab (6 days vs. 4.7 days and 2.5 days) [30, 31]. However, its major side effect was intraocular inflammation, probably due to Escherichia coli fragments in its structure, which occurred in 15-16% of cases according to CEDAR, SEQUOIA and MAPLE trials and 7.5-10.4% in BAMBOO and CYPRESS trials [30, 32, 33]. Abicipar pegol was not approved for use by the FDA in 2020.

Another novel approach to anti-VEGF treatment includes a bispecific antibody blocking VEGF-A and angiopoietin-2 (Ang-2): faricimab (RG7716). It is an agent developed by Roche/Genentech and administered intravitreally [33, 34]. The level of Ang-2 is elevated in retinal pathologies, such as CNV formation [35]. Angiopoietin-2 inhibits the Tie2 receptor, which leads to inhibition of formation of endothelial tight junctions and in effect to leakage in the endothelial cell layer [36]. Thus, blocking Ang-2 results in reducing inflammation and increasing the permeability. The structure of faricimab includes modification of the Fc fragment that reduces the systemic exposure, which lowers the risk of intraocular inflammation [34]. The anatomical effect, visual acuity outcomes and safety outcomes show faricimab to be non-inferior to ranibizumab, as was proved in the STAIRWAY clinical trial. The endpoints in best-corrected visual acuity and anatomical imaging with optical coherent tomography in faricimab therapy were comparable with ranibizumab [29, 37].

Gene therapy in neovascular AMD

Gene therapy is experiencing rapid development, which is proved by introduction of Luxturna in 2017. It is a drug effective in diseases resulting from mutations in the RPE65.

<table>
<thead>
<tr>
<th>Table 1. Summary of main characteristics of selected anti-VEGF agents</th>
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<tbody>
<tr>
<td>Substance name (commercial name)</td>
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<tr>
<td>---------------------------------</td>
</tr>
<tr>
<td>Pegaptanib sodium (Macugen; Eyetech Pharmaceuticals/Pfizer)</td>
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<tr>
<td>Bevacizumab (Avastin; Roche)</td>
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<td>Ranibizumab (Lucentis; Novartis)</td>
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<td>Aflibercept (Eylea; Bayer)</td>
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<td>Brolucizumab (Beovu; Novartis)</td>
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<td>Abicipar pegol (Rayocta; Allergan)</td>
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<td>Faricimab (RG7716) (Roche/Genentech)</td>
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gene, e.g. Leber congenital amaurosis (LCA) and severe early-childhood-onset retinal degeneration (SECORD). Luxturna was the first ophthalmic gene therapy introduced [42]. Gene therapy is also under investigation in other ocular diseases, such as glaucoma, where experimental therapies on a mouse model show promising effects [43].

Due to the fact that anti-VEGF therapy’s effects in AMD do not prove the permanent visual acuity conservation and due to the fact of technical and financial difficulties of anti-VEGF intravitreal injections, there is a strong need to develop gene therapies for nAMD. Authors confirm that the anatomical and immunological features of the human retina make this organ an ideal target for gene therapy [7].

The main way of gene supplementation in ophthalmic research is with the use of either a recombinant adeno-associated viral (AAV) or lentiviral vector delivered preferably subretinally, whereas intravitreal and suprachoroidal administration is also possible [7, 44].

There are two main therapeutic strategies in nAMD: gene silencing and AAV-mediated gene therapy with therapeutic proteins [45].

Gene silencing gene therapy
Gene-specific therapy targets a specific gene responsible for the pathologic process. It uses site-specific endonucleases in order to excise the faulty gene. The type of endonuclease which is gaining popularity is CRISPR-associated (Cas) systems: the bacteria-derived RNA-guided systems [45, 46]. Currently on-going CRISPR-Cas experiments on AMD target VEGF-A, VEGFR2 and HIF-1α genes [47, 48].

Virus mediated gene augmentation therapy
Adeno-associated virus vectors exert low pathogenicity, immunogenicity and capacity for self-replication [45]. The AAV vector carrying pigment epithelium derived factor (PEDF) was proven effective with a dose-related response [49, 50]. Another possible therapy involves sFLT-1 gene, e.g. Leber congenital amaurosis (LCA) and severe early-childhood-onset retinal degeneration (SECORD). Luxturna was the first ophthalmic gene therapy introduced [42]. Gene therapy is also under investigation in other ocular diseases, such as glaucoma, where experimental therapies on a mouse model show promising effects [43].

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Dry AMD
Retinal damage is triggered by factors including aging and environmental stress [5]. Complement is one of the main immunological components in the molecular pathophysiology of the cell degeneration. Complement’s role has been proven crucial in retinal ganglion cell death in glaucoma [62] and its deregulation is also underlined as one of the causes of dry AMD formation [63].

The classical complement pathway is triggered by dead or damaged cells tagged by the C1 complex, which then triggers C3 convertase. In effect, C3 converts into C3a (anaphylatoxin) and C3b (opsonin). C3a activates C5 convertase, which triggers the conversion of C5 into C5a (anaphylatoxin, chemotactic factor) and C5b (the first part of the membrane attacking complex – MAC). MAC formation leads to cell lysis. Complement’s other function is to enable opsonization, which leads to further phagocytosis [63, 64].

Component factor H (CFH) and complement factor I (CFI) are proteins triggering the alternative complement pathway and activating C3 convertase. The complement cascade results in MAC formation.

MAC plays the role of the effector of the complement cascade in cell death. Studies have shown its presence in aging maculae considered healthy. It has been found in higher concentrations starting from the age of 50 as well as in patients with AMD [58].

CFI inhibitors inactivate C3b, preventing MAC formation on the host cell surface [58]. CFH inhibits activation of the alternative complement pathway. Certain types of mutation of the CFH gene result in a higher MAC concentration in the macula and are considered the strongest genetic risk factors for AMD [63, 65, 66]. The effect of MAC on retinal vessels’ epithelium is the loss of choroidal vasculature, which leads to formation of atrophic regions [58].

Complement inhibitor antibodies in dry AMD
Eculizumab is an antibody inhibiting complement component C5 and lampalizumab is an antibody inhibiting complement factor D [67-69]. The primary results of the trials with complement inhibitors raised high hopes for its future impact on dry AMD successful therapy. Lamplizumab, however, did not reduce the enlargement of geographical atrophy in randomized clinical trials [68, 69].

Pegcetacoplan (APL-2) is an anti-C3 antibody. Avacincaptad pegol (Zimura) is an anti-C5 antibody. There are studies reporting significant reduction of the GA growth rate by intravitreal administration of APL-2 and Zimura. However, patients receiving these agents presented 3 to
17 times higher risk of developing nAMD [66, 70, 71]. Another trial examined monoclonal anti-amyloid-β therapy intravenously and proved no significant effect of the agent [72]. Possible future therapies targeting CFH, CFI and MAC are under investigation [73].

Gene therapy in dry AMD

The complement cascade targeted gene therapy was supposed to be effective in dry AMD. However, recently there have been few reports providing significant results of these therapies. The designed gene therapies focused on the complement cascade. One of the agents augmented the expression of protein CD59. Its mechanism protects RPE cells by CD59-mediated inhibition of the membrane attacking complex [74, 75].

Other therapies in dry AMD

Due to the need for more effective solutions in dry AMD treatment, there are many ways to approach the disease under study. Jaffe et al. examined tandospirone administered topically, with no significant result [76]. Lutein together with other antioxidative agents such as zeaxanthin and fatty acids was proven to play a role in retinal protection in AMD [77-79]. A study on emixustat hydrochloride resulted in no reduction of AMD progression [80]. The most recent reports showed no benefits of oral administration of alpha lipoic acid (ALA) on GA formation inhibition [81].

Conclusions

The treatment of AMD became more successful in the recent decades as anti-VEGF treatment became introduced on a large scale. There is more impact put on the prevention of the disease and early diagnosis. Neovascular AMD therapy still lacks long-lasting and easier to administer therapies.

Still, there is a high need for developing new agents preventing macular atrophy formation. The complement cascade inhibitors trials have not been proven effective so far, but still new agents are being examined.

The authors declare no conflict of interest.

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