Targeted therapy in age-related macular degeneration (AMD)

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SUMMARY:

Introduction and purpose:

Age-related macular degeneration (AMD) is a major cause of blindness in highly developed countries, with blindness frequency of 8.7%. This article is a review of the latest therapeutic options for AMD.

A brief description of the state of knowledge:

AMD is a multifactorial disease which etiology is not completely understood. Its development is affected by disorders at the cellular level, environmental and genetic factors. Intraocular injections of anti-VEGF agents are currently considered as the basis of AMD neovascular

treatment. In the search for better and better therapeutic agents, the effects of administration of bevacizumab and ranizumab were tested. Many clinical studies confirm long-term and effective improvement in patients' vision after using the above-mentioned drugs, indicating that the initial response to treatment and the persistence of the therapeutic effect is individually variable and may be associated with genotypic difference. Another promising alternative to AMD treatment is the use of specific viral vectors that transfer substances slowing down the disease into the vitreous. Another method of gene therapy is the use of HIF transcription factors (hypoxia-induced factors), for now, the research is performed on animal models. Patients with dry AMD also have a chance for successful treatment. Examined gene therapy in dry form of AMD, including retinal surgery combined with viral vector injection, is in I/II phase study in Great Britain.

Conclusions:
Looking at the number of blindness cases in highly developed countries caused by AMD, every effort should be made to introduce effective treatment that at least inhibits disease progression. Undoubtedly, more research is needed to confirm the efficacy and long-term safety of AMD.

Key words: age-related macular degeneration; gene therapy; VEGF; genetic vectors

ARTICLE:
Introduction and purpose:
Age-related macular degeneration (AMD) is a major cause of vision loss in developed countries, especially in the geriatric population. The incidence of blindness as a consequence of this condition is 8.7%. Clinically there are two forms of AMD- dry and wet (up to 90% of cases). The second form develops faster and more often leads to total blindness. It is expected that in 2020 the number of patients with AMD will increase to 196 million, and up to 288 million in 2040, due to the aging of the human population [1].

AMD primarily threatens central vision, because the lesions affect the macula. Loss of central vision can make it hard to drive, recognize faces and read. Visual hallucinations may also occur. Over the years changes in the retina lead to the formation of so-called drusen, tiny accumulations of extracellular material; 95% of the population over the age of 43 has such changes. As the disease progresses, retinal pigment epithelial cells (RPE) are become to be hypo- or hyper-pigmented in connection with the appearance of new drusen. This is considered to be early or intermediate stages of AMD. In the more advanced stages of AMD, the dry form is called geographical disappearance (because cell death occurs here), and the wet or exudative form is called the neovascular form of AMD (the primary feature is the growth of abnormal blood vessels). Currently, the majority of therapies are directed at the wet form of AMD. The main therapeutic goal is antibody-based inhibition of growth factor-VEGF. Only small group of patients, who is receiving this therapy, achieves long-lasting results that improve visual acuity without additional, repeated treatment. However, most patients require long-term treatment to slow down the development of the disease or present progression despite therapy [2].

The pathomechanism of changes on the retina appears to be multifactorial. Disfunction at the cellular level in AMD may refer to: photoreceptor, retinal pigment epithelial cells (RPE), Bruch's membrane (BM), or choroid. Observed changes are most likely the result of destabilization of reactive oxygen species (ROS), phagocytosis, extracellular matrix remodeling, and alternative complement-related inflammation [1]. Relevant evidence suggests mitochondrial damage and epithelial cell death in AMD pathogenesis, but the effect of mitochondria and humanin G (HNG) is still not fully understood. It has also been shown that humanitin, which plays an important role in the pathogenesis of AMD, may in the future be a biomarker for early detection of lesions [3]. In the neovascular form, the formation of new, pathologically changed blood vessels is crucial due to changes at the level of VEGF growth factor. That's why the most modern therapies are directed against it.

In recent years there have been new reports and treatment suggestions for patients with AMD, especially with the dry form. This may be a breakthrough in ophthalmology. For sure, the effectiveness and safety of the innovative therapies requires a lot of research and clinical trials.

Description of the state of knowledge:

Etiologically AMD is a multifactorial disease. Among the pathogenetic factors, there are complement disorders, sensitivity to age-related maculopathy, and finally changes at the level of vascular endothelial growth factor (VEGF) and the VEGF receptor axis (VEGFR). The possible involvement of other genetic factors in AMD pathophysiology has also been demonstrated, such as tissue inhibitor metalloproteinase (TIMP), fibrillin, 4A3 collagen and metalloproteinases (MMP). Moreover, it is suggested that environmental factors, i.e. advanced age, female gender, white race, smoking, increased body mass index, hypertension and hyperopia may be a predisposition to AMD. It has also been shown that some biomarkers like carboxyethylpyrrole or homocysteine correlate with the course of AMD. Despite current knowledge and numerous scientific studies in recent years, the etiology of AMD is still not fully understood, which is a challenge for modern medicine.

Currently, the basic treatment of neovascular form of AMD (nAMD) are intraocular injections of anti-VEGF agents, but also photodynamic or thermal laser therapy. The use of intravitreal anti-VEGF injections with pegaptanib has revolutionized the treatment of nAMD- it was the first approved anti-VEGF agent. Because pegaptanib binds to a single VEGF isoform, it was less effective than expected. In the following years, more preparations with the same basic mechanism of action were introduced - bevacizumab and ranibizumab. Both of these molecules bind all VEGF isoforms. Compared with bevacizumab, the immunogenicity of ranibizumab has a 5-20x greater potency due to its greater affinity for VEGF. The latest approved drug for the treatment of nAMD, aflibercept, consists of the VEGF binding domains of human VEGFR1 and VEGFR2 fused to the human immunoglobulin-G1 Fc domain. Aflibercept acts as a „decoy receptor" that not only binds VEGF, but also placental growth factor (PLGF). Regarding dry AMD, treatment is very limited. In clinical practice, almost only palliative antioxidant therapy is used in different forms, which delays progression in 20-25% of patients [1].

The efficacy of using the intravitreal anti-vascular endothelial growth factor (anti-VEGF), bevacizumab and ranibizumab, has revolutionized the treatment of neovascular age-related

macular degeneration (nAMD). In multicenter randomized clinical trials in which bevacizumab and ranibizumab were compared, both drugs seem to provide effective and long-term improvement in patients' vision. However, the results of these studies also show that there is individual variation in the initial response to treatment and in the persistence of the clinical effect. The specific EPAS1 gene may be relevant, because probably affects the response to treatment against VEGF. It is a transcription factor that is mainly present in highly vascularized tissues. [4]. In 2013, Zhao L. et al. examined whether different variants of the vascular endothelial growth factor A (VEGFA) gene are associated with different responses to anti-VEGF therapy. Their results showed a potential pharmacogenetic relationship between the VEGFA gene and response to treatment against VEGF [5]. A similar relationship was noticed a year later by scientists led by Park UC. Who studied the effectiveness of ranimizumab treatment depending on the genotype. Analysis showed that the rs3025039 genotype was associated with a much higher chance of improved vision at 24 months than other genotypes. No polymorphism was significantly associated with the number of intravitreal injections [6]. In other studies, the VWA3A gene variant was associated with a poorer response to anti-VEGF treatment in patients with nvAMD. The VWA3A protein is a precursor of the multimeric von Willebrand factor, which is involved in blood coagulation, a system previously associated with nvAMD [7]. Slightly different results were obtained by scientists under the management of Hagstrom SA in 2015. There was no significant pharmacogenetic relationship between different variants of the VEGFR2 gene, rs4576072 and rs6828477, and response to anti-VEGF treatment in patients with neovascular AMD [8]. Due to a number of evidence of a pharmacogenetic relationship with anti-VEGF drugs, individualized genetic-based therapy can lead to optimal treatment of AMD with neovascularization.

High therapeutic hopes are also available using specific viral vectors that can transfer certain substances, that slow the development of AMD, into the vitreous. In recent years, lentiviral vectors have been discussed for the transfer of endostatin and angiostatin genes. The collected data shows that EIAV (Equine Infectious Anemia Virus) injections are safe and well tolerated also they provide reproducible, persistent transgene expression. Lentiviral vectors, based on EIAV, are platforms for gene exchange or delivery of genes to the eye. Campochiaro's 2017 study also proves that prolonged expression of high doses of endostatin and angiostatin in the eye can reduce fluorescein angiographic leakage, but does not reliably eliminate subretinal fluid and/or internal fluid in patients with advanced exudative AMD. The data provides strong justification for the use of EIAV-based vectors for the treatment of chronic eye diseases [9]. Commonly used viral vectors in gene therapy models also include recombinant adeno-associated virus (rAAV) vectors. The rAAV vectors are found to be the most effective in retinal gene therapy due to long-lasting photoreceptor transduction and RPE. [1] Delivery of various substances directly into the vitreous of the eye using viral vectors will certainly undergo dynamic development in the coming years.

Another potentially effective method of AMD gene therapy may be treatment targeted at HIF transcription factors, i.e. factors induced by hypoxia. Currently, anti-HIF gene therapy studies, related to nAMD, are only conducted in animal models. HIF assessment appears to directly alleviate angiogenesis and inflammatory responses, both associated with the initiation and progression of nAMD. HIF inhibition by gene therapy constructs is most often achieved by

anti-HIF microRNA (miRNA). Expression of miRNA-20b modulates VEGF by targeting HIF-1α and STAT3, which has been suggested as a potential candidate for nAMD gene therapy. Nevertheless, it has been suggested that miRNAs show significant non-specificity by targeting multiple pathways; therefore, the use of HIF-specific RNAi has been reported as potentially beneficial in AMD patients [1].

It has also been shown that lactoferrin, a type of glycoprotein, contained in exocrine fluids such as tears, breast milk, sweat and saliva, applied topically to the eyeball has a therapeutic effect in the mouse model of a dry eye. Studies have confirmed that the administration of lactoferrin had a significant inhibitory effect on HIF in retinal neuronal cells [10].

In 2019 search for new targeted therapies in the AMD studies also focus on genome editing by CRISPR/Cas9 viral delivery, which may include a new treatment option for inherited and acquired eye diseases. The results of these tests require further observation [11].

Interleukin-8 (IL-8) may affect the predisposition to age-related macular degeneration (AMD), but the results of these studies are still controversial and inconclusive. A research group led by Liu J. in early 2020 conducted a meta-analysis of studies that examined IL-8 gene polymorphisms and IL-8 levels in patients with AMD and control. The overall meta-analysis result showed that the IL-8 +781 C/T polymorphism (rs2227306) was significantly associated with predisposition to wet AMD, but no such significant relationship was found for IL-8 -251 A/T (rs4073). NvAMD patients had significantly elevated levels of IL-8 compared to the control group. These studies suggest that the IL-8 +781 C/T polymorphism affects the predisposition to macular degeneration [12].

Recent scientific reports give a shred of hope for a group of patients with dry AMD. So far, their treatment option have been very limited. Professor Robert MacLaren from the University of Oxford conducts the Phase I/II FOCUS study in patients. The examined gene therapy is to counteract the progression of dry AMD by inhibiting the overactive complement system in this disease. Gene therapy in AMD involves retinal surgery and injection of the solution containing the viral vector. The DNA-modified virus infects retinal pigment epithelial cells (RPE). As a result, the genetic defect causing AMD is corrected. If the effects of the therapy prove to be long-lasting, it will be possible to perform the procedure once in a lifetime [13].

Summary:

Age-related macular degeneration (AMD), as it is the most common cause of blindness in developed countries, should be a priority in current ophthalmological research. Every effort should be made to introduce effective clinical tools to treat effectively or at least to slow down progress of this disease, considering the fact that up to 10% of all blindness cases in these countries may be caused due to this macular pathology. Modern scientific achievements seem very promising. Anti-VEGF containing intravitreal injections commonly used in the neovascular form of AMD have proven effective and well tolerated in the light of the available literature.

Promising results also relate to the use of viral vectors,by which specific active substances have been delivered to the vitreous body, e.g. EIAV subretinal injections, or adenovirus-associated recombinant virus (rAAV) vectors. Gene therapy using anti-HIF and microRNA also appears to be a potential candidate for nAMD gene therapy. Not without significance are the results of studies that focus on searching for relationships between environmental or

genetic factors and the risk of developing AMD, as well as factors directly affecting the results of patient treatment. Such relationships have been demonstrated, among others in the case of IL-8 gene polymorphism, the effect of humanin G (HNG), or complement system dysfunction. Noteworthy are also recent reports on new treatments for dry AMD, which have been very limited.

Undoubtedly, further research is still needed to confirm the efficacy and, above all, the long-term safety of current AMD treatment. It is possible that in the era of very rapid development of genetic and molecular sciences, scientists will be able to develop innovative solutions that will allow patients at the early stages of the development of the disease, to maintain eyesight and thus significantly improve their quality of life.

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