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AMD – THE RETINAL DISEASE WITH AN UNPRECISED ETIOPATHOGENESIS: IN SEARCH OF EFFECTIVE THERAPEUTICS

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Abstract: AMD (age-related macular degeneration) is a progressive vision-threatening ocular disease, affecting central region of the retina – the macula – and manifesting in the elderly. AMD is a degenerative disease, and the degeneration affects primarily the retinal pigment epithelial (RPE) cells and secondarily the photoreceptors, leading consequently to disturbances or partial loss of central vision and legal blindness. Clinically, the disease is classified as: atrophic – dry AMD (in majority of cases), and neovascular – wet AMD (with choroidal neovascularization – CNV; 10–15% of all AMD cases). Pathogenesis of AMD is complex, multifactorial and only poorly recognized. Main risk factors include: advanced age, genetic predispositions, environmental determinants, history of exposure to intensive light and smoking. At least four molecular processes contribute to the development of AMD pathology: lipofuscinogenesis, drusogenesis, inflammation and choroidal neovascularization (in wet AMD). Since vascular endothelial growth factor (VEGF) is a predominant proangiogenic factor in CNV, the wet AMD can be treated with intravitreous application of „anti-VEGF" agents (Avastin, Lucentis, Eylea). Till now, there is no approved therapy for dry AMD, although several agents/treatments are currently in clinical trials. This paper briefly describes major molecular and cellular events leading to AMD, and presents currently used and new experimental therapeutic strategies against AMD.

Keywords: AMD, age-related macular degeneration, pathogenesis, lipofuscin, drusen, oxidative stress, inflammation, retina, vision, therapeutic strategies

Age-related macular degeneration (AMD) is one of the most common irreversible causes of severe loss of vision, including legal blindness. In its course the disease inevitably leads to serious compromise of quality of life. AMD affects several dozen millions of people over the age of 60 worldwide, with hundreds of thousands of new cases diagnosed each year. Clinically, the disease is classified into slowly progressing atrophic – dry AMD (~85% of all AMD cases), with advanced or late stage named geographic atrophy (~35%), and rapidly progressing neovascular (exudative) – wet AMD (10–15% of all AMD cases) (1, 2). The mentioned clinically distinct two forms of AMD (dry and wet) have however common molecular and cellular "roots", which are often referred to as the early-intermediate stage AMD or age-related maculopathy (ARM). At early stages, the pathology is developing asymptomatically; however, later on – at certain moment of the disease progression – the patients start to experience some problems with vision (non-specific mild signs decreasing the comfort of vision), which force them to contact an ophthalmologist. With time, the symptoms tend to become more pronounced – in such patients an ophthalmologist, after detecting in addition the presence of drusen in the macula region, as well as hypo- and/or hyperpigmention of the RPE, or the presence of newly formed subfoveal blood vessels originating from the choroid, properly diagnoses the disease as the dry or the wet form AMD – in one eye or both eyes.

As already stated, the wet form AMD, characterized by subretinal extravasations of the choroid-derived neovessels (choroidal neovascularization – CNV) and hemorrhage under and into the photoreceptor cell layer in the macula region, is a rapidly developing and devastating phenomenon diagnosed in relatively small portion of all AMD cases; thus, a question arises as to whether the rapidly developing

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"macular" CNV, manifesting the presence of the wet form AMD, is a complication of the dry AMD, or it represents an independent from atrophic form (including geographic atrophy – GA), macular disease? An answer to this question may have important therapeutic and prognostic implications. Our knowledge concerning AMD pathogenesis, from its early stages to advanced pathology, is still incomplete, which makes problems in proper interpretation of many molecular, cellular and clinical phenomena together with their underlying mechanisms, as well as functional consequences, occurring during the course of the disease. And this is the reason why therapeutic approaches used in the past and at present were/are of limited efficacy, which particularly is the case with atrophic – dry AMD. Since vascular endothelial growth factor (VEGF) is a predominant proangiogenic factor in CNV, the wet AMD can be treated with intravitreous application of „anti-VEGF" agents (Avastin, Lucentis, Eylea). Till now, there is no approved therapy for dry AMD; yet, many chemically different agents showing different mechanisms of action are currently in clinical trials (see last paragraph).

The aim of this article is twofold: 1. to provide current knowledge on AMD pathogenesis, with deeper insight into the early stages of the disease, and the dilemma on real status of the wet-neovascular AMD *versus* the dry-atrophic form, and 2. to summarize latest efforts on potential therapeutics for AMD, with emphasis on therapies for the dry form AMD.

AMD – non-modifiable and modifiable risk factors

There are many various risk factors that may predispose or facilitate the pathogenesis of AMD. Such factors are usually classified as non-modifiable and modifiable ones. The former group includes genetic predispositions and age, while the latter group includes a number of environmental, behavioral or other factors that possibly may, to varying degree (depending on individual characteristics and sensitivity), influence molecular mechanisms or cellular processes underlying the disease development (3).

Genetic risk factors

Genetic predispositions seem likely to occur, as much evidence points to a familial component of AMD. Several genes were earlier identified that cause diseases with clinical features that overlap with AMD, e.g., *ABCA4*, *ELOVL4*, *FIBL-6*, *APOE*, *SOD2*. Although mutations in the mentioned genes may to some extent contribute to the development of particular features of AMD, they obviously are not responsible for the advanced and complex AMD pathology. In 2005, the pioneering discovery of complement factor H (*CFH*) as a major AMD susceptibility gene showed that there is a gene, whose polymorphism (Y402H), leading to increased activity of the complement system, likely contributes to the AMD pathology. Further extensive investigations, including genome-wide association studies (GWAS), have confirmed a number of additional genetic risk loci – a total of 20 susceptibility loci, which considered together can explain up to 60–70% of the disease heritability (4). Based on common SNP (single nucleotide polymorphism) associations, two loci seem to contribute to the greatest AMD risk: 1q31 and 10q26, the former related to already mentioned Y402H variant in the *CFH* gene encoding CFH (a negative regulator in the alternative complement pathway), the latter representing non-complement related genes involved in AMD, i.e., *ARMS2* (*age-related maculopathy susceptibility 2*) / *HTRA1* (*high-temperature requirement A serine peptidase 1*) (5). At present, we know that a number of other genes within the alternative complement pathway may be associated with AMD in both a non-protective and protective manner, these include genes encoding CFI, C3, C2/CFB, C7 (4, 6).

Aging as a risk factor

Aging favors the development of AMD, particularly in predisposed individuals (due to concomitant presence of some additional risk factors). The term "age-related" in the disease name – AMD is fully justified, as the age is the major (albeit individual, i.e., specific to each subject) and unavoidable determinant of various dysfunctions at the cellular and organ level. The term "age-related physiology" covers several changes or dysfunctions that may appear important for AMD pathology, such as: extracellular drusen formation, Bruch's membrane stiffening (including increasing thickness of Bruch's membrane and lipid accumulation), intracellular debris (resulting from oxidative stress-induced cell/tissue damage, lipofuscin, advanced glycation end products – AGEs), mitochondrial defects, cell loss and tissue degeneration. Some sort of age-related changes may be associated with the deficiency of necessary microelements/nutrients or with the loss of function in the aging cells – particularly postmitotic, i.e., non-regenerable cells as, for example, the retinal pigment epithelium cells – RPE or photoreceptors. While the lacking microelements/nutrients

may be supplied from the outside, thus attempting to compensate for their deficiency in particular cells/tissues, we are unable until now to stop the systemic aging process. Considering AMD pathology, it is however important to precisely differentiate between age-related "physiologic" changes from already pathologic events/symptoms (7) – such task may sometimes be really difficult to perform, as in patients with a risk of AMD development, physiology, including that related to aging, seems to smoothly shift into pathology.

Modifiable risk factors

There is an array of environmental and behavioral factors that may increase the risk of AMD development, their role(s) being likely dependent on individual sensitivity and functional tissue/organ characteristics; among them are:

- cigarette/tobacco smoking – this has been identified as the most consistently reported modifiable risk factor for the development of AMD, and increasing the risk of progression to advanced AMD (8, 9).
- light (exposure to intensive light in the past) – depending on environmental lighting conditions, each day the human retina absorbs approximately 10^{12} to 10^{15} photons. Although perception of light (visible spectrum: 400–700 nm) by retinal photoreceptors is a physiological process, excess of visible light, especially in the range of shorter wavelengths, may show toxic effects (10). The blue region (400–500 nm) of the visible spectrum is of particular importance since it has a relatively high energy and can easily penetrate ocular tissues, including the neural retina with photoreceptors (11). In addition, ambient radiation from the sun or from artificial light sources contains varying amounts of UV irradiation from A to C range (220–400 nm). The shorter the wavelength, the greater energy and therefore the greater the potential for photochemical cell/tissue damage. However, although the longer wavelengths are less energetic, they can penetrate the eye more deeply (12). Concerning possible toxic effects: brief exposure to bright light can cause immediate thermal injury, whereas exposure to light for an extended period of time may lead to photochemical damage, including RPE monolayer disruption (10, 11).
- improper diet (fatty, vegetable-poor) – specific dietary recommendations are therapeutically crucial in many ailments and diseases (e.g., diabetes, arterial hypertension); this may also apply to AMD, however the expected preventive/therapeutic results in AMD patients may be poor and varying, despite the fact that there is a vast literature on that topic suggesting the importance of a proper diet or dietary supplements (13). Dietary supplements, containing macular pigments (lutein and zeaxanthin), selected vitamins (E and C) and/or metal salts (zinc, selenium) – all directed to prevent or fight oxidative stress, are widely marketed as a strategy for AMD, but clinical data are inconclusive, giving no firm clues concerning their real efficacy against the disease (13–15).

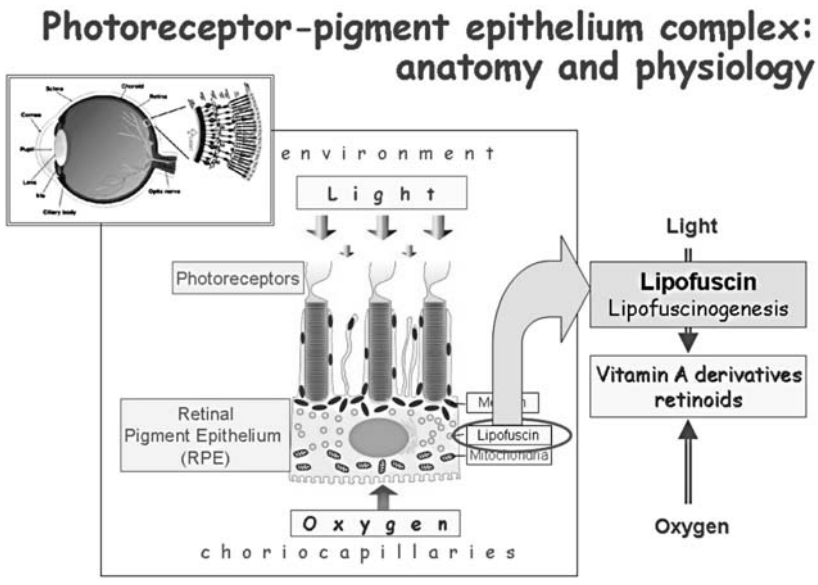

Figure 1. Physiologic aspects of photoreceptor-pigment epithelium (RPE) functional complex. For explanations see text

- Hypertension – possible, but less defined risk factor.

AMD – the origin and development of the pathology

Although the role of at least five processes (oxidative stress, lipofuscinogenesis, drusogenesis, inflammation and neovascularization in wet AMD) in AMD pathogenesis is commonly accepted, the onset of the disease remains elusive since the mechanism triggering the pathology remains unknown. It is practically impossible to detect any sign/change at the very beginning of AMD since, as mentioned earlier, the disease develops inconspicuously over many years. In order to understand the arising pathology it is reasonable to enter physiological events underlying vision, and to analyze those processes whose course may be deteriorated either spontaneously or as a result of the action of at least some risk factors. Figure 1 depicts anatomy and physiology of the photoreceptor-retinal pigment epithelium (RPE) complex, showing also possible propathogenic mechanisms related to light exposure and oxygen supply.

AMD is a slowly progressing disease of degenerative characteristics, with meaningful clinical symptoms occurring many years later, usually in the elderly (60+ years) population. The evidence shows that the degeneration affects primarily the retinal pigment epithelium (RPE) cells and secondarily photoreceptors; early changes in the Bruch's membrane and the choroid are also possible.

The AMD pathology takes place within functional anatomic complex of the macula region embracing: photoreceptors, the retinal pigment epithelium cells (RPE), the Bruch's membrane and the choroid.

Photoreceptors and RPE cells. Physiological roles of the last three structures listed above are to create optimal conditions for work and activity of photoreceptors (rods and cones), whose principal role is to absorb photons of light and thus detect visual signals from surrounding environment. RPE cells, forming monolayer, are in tight contact with photoreceptor inner segments (PIS) containing densely packed molecules of visual pigments – their function is crucial for activity and survival of photoreceptors. RPE cells are multifunctional and take part in the blood-retina barrier structure and function, delivery of nutrients (including oxygen) to photoreceptors, collection of metabolic products from photoreceptors, the role – together with photoreceptors – in the visual (retinoid) cycle, phagocytosis and enzymatic metabolism of constantly shed apical fragments of PIS during the vision process. All the mentioned RPE functions are interrelated and indispensable for maintaining photoreceptors' physiology (see Fig. 1).

The Bruch's membrane is built from 5 layers containing collagen and elastin; its role is to physically separate neural retina from vascular bed consisting of choroidal microvessels. Changes in the structure and function of the Bruch's membrane can facilitate and underlie the CNV phenomenon.

The choroid represents one of the two circulatory systems delivering blood to the retina; the choroid or choroidal system, *via* its choriocapillaries, is responsible for supplying the outer third part of the retina (containing photoreceptors and RPE cells; which physiologically remains completely avascular) with necessary microelements and oxygen.

It seems highly unlikely that there is only one signal/mechanism responsible for initiation of the pathology. A widely accepted scenario takes into consideration an interactive role of two, three, or even more factors. Thus, due to interplay of plurality of risk factors, both endogenous and exogenous or behavioral, which might predispose, or even contribute to the development of the disease, some important physiological processes underlying vision may become irreversibly deteriorated. In other words, the critical physiological processes responsible for the vision process (including the visual excitation cascade) move beyond the limits of homeostasis, creating a biochemical platform for the future pathology. This involves: uncontrolled and non-neutralizable oxidative stress, intensified lipofuscinogenesis in the retinal pigment epithelium (RPE) cells, formation of drusen under the RPE monolayer (i.e., in the direction of Bruch's membrane) – the process called drusogenesis, low grade chronic inflammatory process known as parainflammation, and enhanced activity of the complement alternative pathway (as a result of e.g., Y402H mutation in *CFH* gene); some additional less recognized factors/phenomena may also play in concert in building future pathology (16–19).

RPE – the primary site of AMD pathology

Our bodies contain many non-regenerable (postmitotic) cells, particularly within the central nervous system. These include the photoreceptors (only the external segments that contain photopigments, i.e., photoreceptors outer segments (POS), are subject to regeneration) and retinal pigment epithelium (RPE) cells. In the pathogenesis of

AMD, RPE cells are the first cells that become metabolically inefficient and thus undergo degeneration; dysfunction and atrophy of photoreceptors is secondary, as they are unable to function and survive without functionally efficient RPE cells, and therefore also undergo degeneration. The pathological process involves mostly a small region of the retina, known as the macula, where the cone photoreceptors, responsible for acute and color vision, are predominant. Therefore, first clinical symptoms of AMD include several nonspecific symptoms resembling malfunction of the vision process, e.g., blurred vision, defects with central vision of various intensity or gradual loss of color vision, "warping" of perceived images (1, 20).

When the eyes are open, photoreceptors are continuously working, absorbing light photons and "recording" the image of the environment. In other words, eyes permanently record this image in an automatic fashion, generating the first signal of a complex, multisynaptic vision process. POS, filled with visual pigment molecules, are characterized by significant functional dynamics; they wear off upon continuous function and, as a consequence, the apical fragments are constantly shed and captured by neighboring RPE cells. At the same time, POS are being rebuilt in order to maintain appropriate size (which is an important parameter determining the efficacy and survival of photoreceptors). Regeneration proceeds from the perikaryon, i.e., the photoreceptor inner segment (PIS), and requires numerous building blocks, including docosahexaenoic acid (DHA). These building blocks are supplied by the RPE cells and originate partly from the captured POS fragments and partly from circulation (consumed food) (19).

One of the many important roles played by the RPE cells is "digestion" of the absorbed (and continuously being absorbed) photoreceptor material stored in phagolysosomes. Despite the fact that phagocytosis and enzymatic degradation occurring as a result of the activity of numerous lysosomal enzymes and physiological processes that had developed over thousands of years in creatures dwelling on Earth and making use of the visual organ system (retinal processes that govern visual perception in many vertebrates, including humans, are generally similar), they seem to be of limited efficacy, at least in humans. This claim is supported by systemic accumulation of lipofuscin, known as the age pigment, in RPE cells (Fig. 1).

Lipofuscinogenesis – age-related physiology or pathologic event?

Lipofuscin and the process of its formation, i.e., lipofuscinogenesis, are not the attributes of RPE

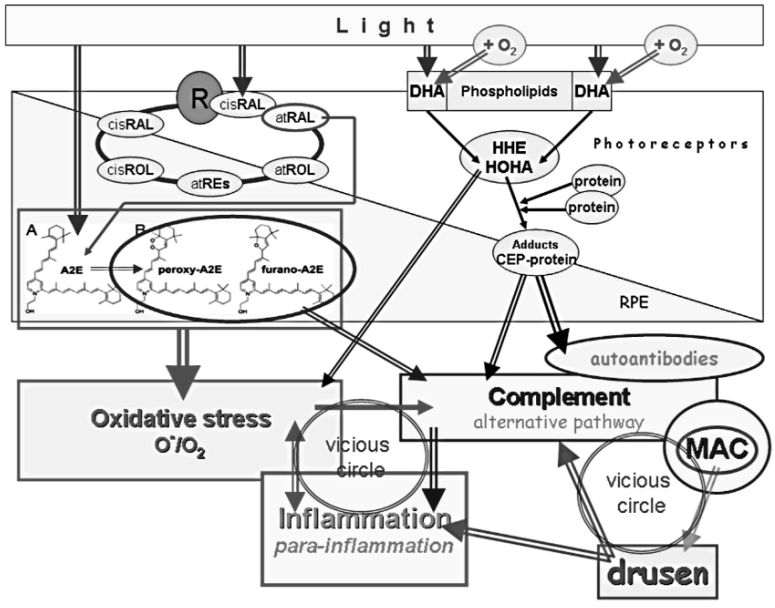

Figure 2. Interrelations between molecular and cellular processes occurring at early-moderate stages of AMND pathogenesis. For explanations see text. Note that some mechanisms may form a kind of vicious circuses. Abbreviations: RAL – retinal, ROL – retinol, at-RAL – all-trans-retinal, atREs – retinyl esters, DHA – docosahexaenoic acid, HHE – 4-hydroxy-2-hexenal, HOHA – 4-hydroxy-7-oxyhept-5-ene acid, MAC – membrane attack complex

cells and connections between photoreceptors and RPE, as they are also present in other non-renewable cells, such as neurons, cardiomyocytes or skeletal muscle cells. Lipofuscin, being accumulated in various cells in the course of aging, is also known as "age pigment". As such, lipofuscinogenesis, together with accumulated lipofuscin, could be considered as events lying within frames of physiology or "extended" or age-dependent physiology.

However, there is an important compositional difference between the retinal lipofuscin and the pigment accumulated in other body cells. The retinal lipofuscin has a unique characteristics because of the presence of retinoids (vitamin A derivatives), originating from the visual cycle, particularly bisretinoids – products of spontaneous fusion of two molecules of all-*trans*-retinal, generated *via* photoreaction (i.e., by absorption of photons) by 11-*cis*-retinal, a cofactor of the visual pigment. Taking into account that bisretinoids are photocytotoxic compounds, an important issue deals with conditions promoting their formation – do they represent "extended" (and likely reversible) physiological reactions, or they, after reaching certain level of activity, direct pathological pathway/s? (see Fig. 2). In order to answer such questions, it is necessary to follow reactions/pathways underlying first steps of vision physiology.

The cis→trans retinal transformation is the crucial first step of visual cycle, initiating further conformational transformations of opsin (i.e., the visual pigment protein) into its active forms (e.g., meta-rhodopsin II), capable of progressing the visual cycle with the final effect consisting in the closure of cGMP-dependent cation channel in the cellular membrane of photoreceptors and quenching the so-called darkness current. In this time, the cellular membrane of photoreceptors is hyperpolarized only to regain the state of being ready to absorb another photon, i.e., the depolarization state; the active pigment, capable of absorbing photons, is a molecule, e.g., rhodopsin, that contains a light sensitive co-factor, 11-*cis*-retinal (21).

The all-*trans*-retinal (at-RAL) formed, following photon absorption, is completely dissociated from the visual pigment and undergoes further physiological transformations in the retinoid cycle that takes place in both photoreceptors and RPE cells (Fig. 2). However, part of at-RAL that does not bind the ABCA4 (ATP-binding cassette [transporter] A4 type, also known as ABCR), transporting the retinoid into the sites with all-*trans*-retinal dehydrogenase activity, "falls out" the cycle and spontaneously dimerizes (using often ethanolamine as a "linker") into phototoxic bisretinoids, including A2E (N-retinylidene-N-retinylethanolamine) (Fig. 2). Till now, at least 25 bisretinoid fluorophores originating in photoreceptor cells and resulting from reactions of all-*trans*-retinal have been indentified (22, 23). A2E, which is the best characterized lipofuscin component, seems to represent a thoroughly established stress-inducing product (24–26). Among various biological features of A2E is its, and especially furano- and peroxy- metabolites, significant ability to activate the complement system (an alternative pathway) (27, 28), which is an effector mechanism of the innate immunity, capable of efficiently and automatically acting in system's defense, including destruction of "own" cells (29–31).

Accumulation of lipofuscin deposits in RPE cells is a hallmark of aging in the eye, and in fact is manifestation of metabolic inefficacy of their lysosomal compartment, characterized by reduced autophagy (32–34). The reason for this inefficacy remains unknown, although considering molecular complexity of autophagic processes, the reasons may be multiple, including hypofunction or insufficient quantity/activity of lysosomal enzymes – cathepsins being most predominant in normal conditions. Lipofuscin deposits accumulate with age, and the adverse effects of accumulating products of oxidative stress that accompanies lipofuscinogenesis are intensified. Local inflammatory reaction that develops at certain moment, being manifested by an atypical process referred to as para-inflammation, drusogenesis (drusen, pesudodrusen), and peroxidation of polyunsaturated fatty acids (PUFAs) and PUFA-derived oxidative protein modifications, become the driving forces of the developing AMD pathology (Figs. 2 and 3).

Recently, however, using imaging mass spectrometry, a lack of correlation between the spatial distribution of A2E and lipofuscin fluorescence in the human retinal pigment epithelium (RPE) has been reported (35), an observation rising some doubts on the role of this bisretinoid in increasing lipofuscin fluorescence observed in the central RPE with aging. This potentially important observation needs further verification and, when confirmed, requires rethinking the A2E in relation to both aging and AMD retina.

Long-chain PUFAs – oxidative modifications and formation of carboxyalkylpyrrole-protein adducts

Fatty acids are present in the most diverse forms of life and perform important functions as lipid components in the structure of the

plasmatic/cellular membranes, being responsible for e.g., membrane fluidity, and involved in metabolic/signaling processes; they are important sources of energy and precursors of signaling molecules (including pro-inflammatory, anti-inflammatory, vasoactive, and many other mediators). The vast family of fatty acids comprise saturated and unsaturated compounds, the latter containing one or more double bonds between carbon atoms, C=C, in a hydrocarbon chain, referring to, respectively, monounsaturated or polyunsaturated fatty acids (PUFAs). Long-chain PUFAs include such compounds as: arachidonic acid (AA or ARA; four C=C bonds), eicosapentaenoic (EPA; five C=C bonds), or docosahexaenoic acid (DHA; six double C=C bonds).

PUFA residues of membrane phospholipids are very sensitive to oxidation and the action of reactive oxygen species (ROS), the process known under the term of "lipid peroxidation". Peroxidation of highly unsaturated lipids (e.g., AA, EPA, DHA) leads to complex mixtures of harmful products, including malondialdehyde, acrolein, 4-hydroxy-2-nonenal, 4-hydroxyhexenal, as well as a number of hydroxy-ω-oxoalkenoic acids. The latter compounds, together with their derivatives of the carboxyalkylpyrrole type, are produced in the central nervous system, including the retina. Although structural unsaturation predisposes long-chain PUFAs for peroxidation with resultant harmful compounds, it should be emphasized that these unsaturated fatty acids play many important physiological roles, which, ironically, are related just to their structural unsaturation.

Photoreceptor cell membranes contain exceptionally large amounts of long-chain PUFAs in general, and particularly the most unsaturated docosahexaenoic acid (DHA). It is this high unsaturation which makes DHA particularly susceptible to spontaneous peroxidation and fragmentation into cytotoxic compounds, e.g., 4-hydroxy-2-hexenal (HHE) and 4-hydroxy-7-oxyhept-5-enoic acid (HOHA), the latter being a member of hydroxy-ω-oxoalkenoic acids. HOHA may additionally fuse with protein molecule (e.g., albumin) to form immunogenic carboxyethylpyrrole-protein adducts, e.g., CEP-albumin adduct (Fig. 2) (23, 36–39).

It has been shown that CEP-protein adducts are formed more abundant in ocular tissues (drusen, Bruch's membrane) from AMD patients than from normal human donors, an observation suggesting their role in the pathogenesis of AMD (36, 40). CEP immunoreactivity was detected not only in human retina, but also in human plasma, with values being again significantly higher in the plasma of AMD donors than in the plasma samples of healthy donors (41). Interestingly, the plasma CEP immunoreactivity positively correlated with CEP autoantibody titer (41), indicating that CEP behaves as an antigen

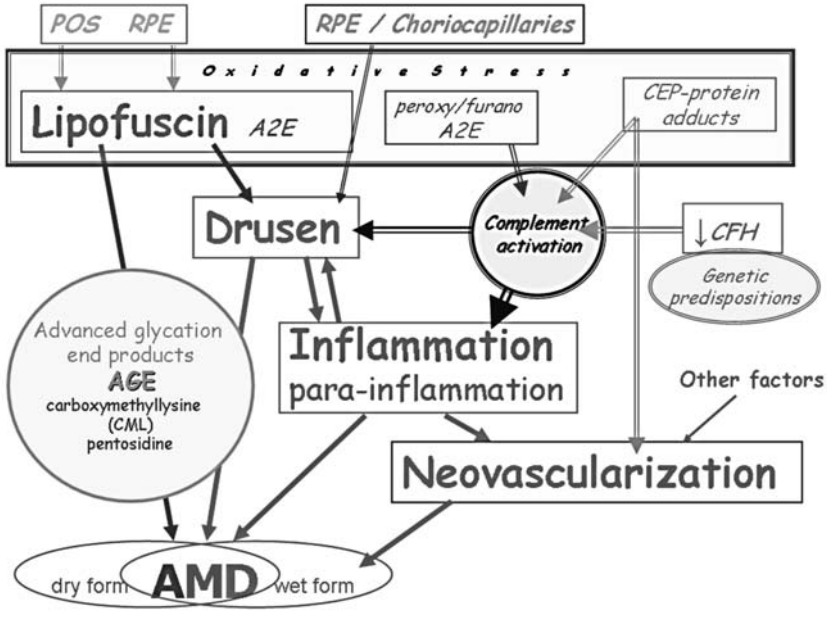

Figure 3. Principal molecular events underlying the development of AMD pathology. For explanations see text

which generates production of specific anti-CEP antibodies (Fig. 2). The immune-mediated events related to immunogenic CEP-protein adducts, which in AMD patients are probably generated through many decades, may contribute as one of many molecular links to the development of AMD pathology. Although direct evidence of the role of CEP-protein adducts in AMD pathogenesis in humans is lacking, recent experiments carried out on mice immunized with CEP-modified mouse serum albumin (CEP-MSA) and Freund's adjuvant (used in an attempt to rise the level of sensitivity to endogenously generated CEP) have shown that the retinas of such animals produced changes similar to those seen in retinas of AMD-suffering peoples; they included: accumulation of drusen, swollen Bruch's membrane, fixation of complement-C3d product in Bruch's membrane, lesions in the RPE cells, decreased electrophysiological responses to light (42, 43). In addition, in mice with laser-induced rupture of Bruch's membrane (an accepted experimental model of CNV), subretinal injection of CEP-MSA significantly augmented CNV, the effect being similar to that produced by injections of VEGF, a major proangiogenic factor (37).

Concerning oxidative stress and the retina, one should not forget that the supply of oxygen (and microelements/nutrients) *via* the choriocapillary system into photoreceptors-RPE cells complex is one of the largest in primates. Taking into account the functional specificity of the retina, particularly of photoreceptors (photosensitivity, extensive metabolism, high partial pressure of oxygen being the substrate for the formation of oxygen-derived radicals), one may suspect that the retina is particularly well predisposed for formation of ROS (39).

The nature must have predicted the potentially adverse, propathogenic processes, such as oxidative stress or lipofuscinogenesis, in the retina, as the tissue, and particularly the macular region – very important for acute and color vision in primates – has been equipped with an array of antioxidative defense systems, including specific macular pigments (MPs): lutein, zeaxanthin and meso-zeaxanthin (44).[1]

Proper functioning of retinal antioxidation defense systems is believed to avert potential early propathogenic changes that may lead to AMD. These propathogenic changes are in fact physiological reactions that become functionally (chemically) impaired due to their intensity and the accompanying overproduction of ROS, crucial transformations of "visual" retinoids into byproducts – bisretinoids, and peroxidation and fragmentation of long-chain PUFAs (Figs. 2 and 3). This may be generally due to indisposition of the aging body – its organs, tissues and cells, and hypofunction of the aforementioned antioxidative defense systems, both enzymatic and non-enzymatic. Since these systems are dependent on the supply of exogenous nutrients, thus their activity is diet-dependent (13) – hence the idea of the role of diet and dietary supplements as preventive/therapeutic modalities for AMD (read further).

Drusen and drusogenesis

Another pathogenic component of AMD are drusen that are amorphous deposits accumulating extracellularly in the area between RPE and the inner collagenous zone of Bruch's membrane (1, 45–47). Drusen are considered the hallmark of AMD (1, 20). Clinically, they are divided into two main phenotypes, "hard" and "soft", depending on their relative size and shape. Although a few small (< 63 μm) hard drusen can be found in at least 95% of the aged populations (1), the presence of numerous larger (≥ 125 μm) hard drusen, and especially soft drusen (≥ 125–250 μm) in the macula is considered – particularly when accompanied by pigment irregularities or depigmentation – a major visible risk factor for developing the advanced forms of AMD. In fact, it was shown that degenerative changes, with either impending or executed photoreceptor cell death, occur in populations of photoreceptors overlying drusen (with a tendency to extend laterally to drusen) of all sizes, including even small subclinical structures (< 63 μm) (45, 46).

[1] It should also be mentioned that the classical antioxidative systems present within the body are classified as either enzymatic or non-enzymatic. Enzymatic systems include three basic enzymes: superoxide dismutase (SOD), catalase and glutathione peroxidase, which are dependent on metal ions, such as zinc, copper, manganium or selenium. The non-enzymatic system consists of carotenoids (including the mentioned macular pigments – lutein, zeaxanthine, meso-zeaxanthine), vitamins E and C and glutathione. The enzymatic system is endogenous; however, the metal ions that are required for its proper function are exogenous, i.e., must be supplied with food. As far as the non-enzymatic is concerned, only glutathione is an endogenous antioxidant. The remaining elements of the system, i.e., carotenoids and vitamins E and C are exogenous factors supplied with food or appropriate dietary supplements. The role of carotenoids is to neutralize singlet oxygen and reactive free radicals. What's interesting, the mechanism underlying these two activities of carotenoids are different and neutralization of free radicals may, in certain conditions (e.g., those when excessive amounts of radicals are present), change the antioxidative activity of these compounds into prooxidative activity.

Drusogenesis is a complex and multifactorial process taking place slowly over many years, and the idea is that the negative impact of the forming drusen on overlying (and neighboring) RPE-photoreceptor cells relies not only on the physical displacement of the RPE monolayer and photoreceptors by them, but also on their indirect influence, most probably *via* the activation of the immune system and local inflammation (48). Proteomic and immunohistochemical analysis of drusen has revealed many protein constituents, including – in addition to RPE remnants – a number of immune-associated elements/molecules, such as dendritic cell processes, immunoglobulins, class II antigens, and components of the complement cascade, e.g., activators, inhibitors (notably CFH), activation-specific complement fragments, and terminal pathway components, including the membrane attack complex (MAC; C5b-9), the latter being lethal not only to foreign pathogens but also to local host cells and tissues (such as RPE, photoreceptors, and other ocular structures) (36, 48, 49).

The complement system is the key element of the innate immune system in host defence (50, 51). It seems likely that local inflammation and activation of the complement cascade, with uncontrolled generation of MAC, may actively contribute to drusogenesis, RPE/photoreceptor degeneration, and Bruch's membrane disruption (associated with neovascular – wet AMD) (17, 47). And *vice versa*, at least some material collected in drusen is endowed with immunogenic properties (e.g., CEP – the lipid peroxidation product), which activate T-cells and macrophages, leading thus to stimulation of the immune responses of both innate and adaptive immune systems that in AMD work in concert (31, 52).

Although drusen constitute an established link in the pathogenesis of AMD (drusen are in fact a hallmark of AMD), they alone do not seem to be sufficient to drive the AMD pathology.

Inflammation

Although the role of inflammation in the pathogenesis of AMD is widely accepted, obviously AMD is not a classic inflammatory disease (31, 53, 54). The state that resembles in some aspects inflammation and may apply to the process taking place in AMD neurodegenerative pathology is named parainflammation (55). This process is thought to be a tissue response to noxious stress induced by various stressors, including oxidative stress. It usually has slow course and characteristics of low grade chronic process which intensifies with age (56). Inflammation – parainflammation plays in concert with the complement system (54).

In consequence, parainflammation, together with microglia activation and macrophage infiltration, as well as activation of the complement cascade, especially alternative pathway, with uncontrolled generation of MAC, may actively contribute to AMD development by increasing drusogenesis, RPE/photoreceptor damage, leading to their degeneration, and Bruch's membrane disruption (associated with the late-stage neovascular AMD).

Lipofuscinogenesis, oxidative stress, drusogenesis, PUFAs peroxidation-fragmentation and inflammation – how far to AMD?

All the mentioned processes – lipofuscinogenesis, oxidative stress, drusogenesis, PUFAs peroxidation-fragmentation and parainflammation – can be formed in different tissues to varying extent even in physiology, and more intensively in age-related physiology (without obvious pathological symptoms); however, their pathogenic potential reveals when they are overactive for years and their products formed in excess, and/or in the presence of some additional factors favoring or strengthening propathogenic mechanisms.

The retina lipofuscin contains an array of visual/retinoid cycle-derived retinoids (which are absent from other age pigments – lipofuscins accumulated in e.g., neurons, cardiomyocytes, skeletal muscle cells), and just their presence, and especially the presence of various bisretinoids (e.g., A2E) endowed with photocytotoxic potential, renders the ocular age-pigment (lipofuscin) into a group of endogenous material with a high pathogenic risk. The process of lipofuscinogenesis is tightly linked with oxidative stress, the latter having an impact on PUFAs peroxidation and fragmentation, as well as formation of immunogenic carboxyalkylpyrrole-protein adducts that accumulate in drusen and blood. Thus again, concerning PUFAs-derived oxidative protein modifications, the retina, due to intensive supply of oxygen, high levels of light exposure, and distinctly high levels of a given fatty acid such as most unsaturated DHA, results in its natural predisposition to ROS generation and PUFAs peroxidation. These phenomena, together with chronic low grade inflammation (parainflammation) related with activated immune system and drusen-containing ingredients with different propathogenic – immunogenic potential, are prone to generate pathologic responses, whose intensity rises with aging, culminating in the gradual appearance of clinically-relevant visual symptoms typical for early-intermediate stages of AMD.

Interestingly, there are more and more experimental data which strengthen the role not only of innate immunity, but also of adaptive immunity in AMD development (52, 54, 57–59). A question whether AMD should be renamed into autoimmune macular disease becomes a vivid issue (59), as genetic predispositions include genes, whose SNPs lead to enhanced immune responses (6) – the risk factors that may be active from the very beginning of our existence.

Choroidal neovascularization

The presence in the macula region of subfoveal newly formed blood vessels originating from choroidal circulatory system (choroidal neovascularization – CNV) in AMD patients is nearly automatically diagnosed as the wet AMD. CNV is an example of neoangiogenesis or angiogenesis and is a process of blood vessel formation (angiogenesis) based on existing vessels.

Blood vessels formed during vasculogenesis, mainly in embryonic life, do not undergo further growth but are stable. The processes which require periodic blood vessel reconstruction, e.g., menstruation, placenta formation or changes in the mammary gland during lactation, form an exception – they represent physiologic neoangiogenesis. Another type of physiological angiogenesis is the formation of vessels in a damaged tissue/organ requiring repair (e.g., wound healing). Neoangiogenesis may also occur as an unwanted phenomenon – pathologic angiogenesis, as is the case for example during tumor growth, or its occurrence in physiologically avascular tissues, such as cornea, vitreous body or the retina macula region. Principal mechanisms underlying physiologic and pathologic angiogenesis are generally similar (although some differences is vessel quality may exist); different may be causing factors, although hypoxia is a major direct proangiogenic condition. A simplified sequence of hypoxia-driven angiogenic pathway may be as follows: hypoxia →HIF-1 (hypoxia-inducible factor) → [gene expression] VEGF-A (vascular endothelial growth factor) → VEGFR-2/VEGFR-1/NRP-1 driven signaling → angiogenesis.

Neovascularization results from a functional dominance of angiogenic factors (e.g., vascular endothelium growth factor – VEGF) over antiangiogenic/angiostatic factors (e.g., pigment epithelium growth factor – PEDF), despite whether the primary event is an increase of angiogenic activity or a decrease of antiangiogenic activity. Molecularly, angiogenesis-neovascularization is a highly complex process involving numerous specific molecules, receptors and signaling pathways (60, 61). Many of them have been recognized and some are targets for modern therapeutics.

Regarding CNV in AMD, an initiation of subfoveal process of neovascularization originating from microcapillary blood vessels of the choroid is still unclear, particularly when the causing factor(s) and primary molecular/cellular events are concerned (62–64).

One of the postulated proangiogenic mechanisms may be linked to the existence of drusen between RPE monolayer and Bruch's membrane. The growing number and size of drusen, as well as the appearance of soft drusen, may lead to local detachment of RPE from Bruch's membrane and choriocapillaries (the latter supplying blood, oxygen and nutrients to external retina layers) and, in consequence, to the appearance of local hypoxia. Hypoxia subsequently provokes the expression of hypoxia-inducible factor-1α – HIF-1α, which, after binding with HIF-1β, becomes an active molecule (HIF-1) capable of promoting expression of several genes, including gene encoding VEGF-A family of proangiogenic factors stimulating the initiation of choroidal neovascularization – CNV (wet AMD).

Oxidative stress and chronic low grade inflammatory process – parainflammation (both taking part in the AMD pathogenesis) may also contribute to the initiation of CNV, because in the course of these processes many factors of various biological activity are expressed, including proangiogenic factors, e.g., VEGF-A (but not only). It is worth of mentioning that recently published experimental data have documented that oxidative stress is in fact able to promote ocular neovascularization (65).

Experiments carried out on mice with laser-induced rupture of Bruch's membrane (an animal model of CNV) have shown that subretinal injection of CEP-MSA (mouse serum albumin) significantly augmented CNV (37). Further experiments utilizing the "human" adduct CEP-HSA (human serum albumin) have documented angiogenic properties of the adduct in two widely used angiogenesis model systems, namely the chick chorioallantoic membrane and rat corneal micropocket assay. The obtained results showed CEP-HSA to be highly potent (active in picomolar amounts) inducer of neovascularization that utilized VEGF-independent pathways (37). While VEGF realizes its action *via* specific VEGF receptor-mediated signaling pathway, CEP-induced angiogenesis probably involves activation of toll-like receptor type 2 (TLR2) (66).

The mechanisms (depicted above) possibly underlying the initiation of CNV in AMD patients

strongly suggest that the CNV phenomenon can be considered a complication of the dry-atrophic form AMD (see Fig. 3).

The cited observations referring to VEGF-independent CNV may have important practical consequences, since CNV occurring in wet AMD is currently treated with anti-VEGF drugs (read later). Yet, CNV resistant to anti-VEGF therapy is not unusual in AMD patients, indicating in such cases the role of VEGF-independent mechanism(s). Therefore, it is not unlikely that in such VEGF-independent CNV in AMD patients, CEP oxidative protein modifications and TLR2-directed signaling pathway may operate – a suggestion that is possible (based on animals' data), yet requiring experimental support for its validity in humans.

Detailed molecular and clinical analysis of and discussion on ocular neovascularization, including CNV, is presented in recently published articles (60, 61, 63).

Therapeutic approaches

Although the symptomatology of AMD is relatively straightforward, there is evidently many various pathogenetic factors, and thus complex mechanisms underlying the disease. Some mechanisms lie within frames of aging physiology, some touch the level of pathology, and some represent already irreversible pathology – everything with a tendency to increase its activity as a function of age. Furthermore, not all molecular links in the disease pathogenesis are recognized. For these reasons, available therapies are not causal treatments but, generally, they help to avoid or, what happens more frequently, to retard further vision loss rather than to substantially improve vision. None of up-to-now used treatments can definitely "cure" the disease or reverse its course. The use of anti-VEGF agents in patients with the wet form AMD may be considered a therapeutic breakthrough, due to their positive effects on quality of vision at least in some patients, yet currently much effort is still done in searching new more effective approaches. The situation concerning therapy of the dry – atrophic AMD is really depressing, as there is no approved strategy for this condition; however, an intensive research to find acceptable remedy is in progress.

Wet – neovascular AMD

Photodynamic therapy

Not far ago, in 2000, an approval of the photodynamic therapy (PDT) using an intravascular photosensitizer verteprofin (a benzoporphyrin derivative monoacid, BPD; Visudine) and low energy visible red laser (689 nm), has at that time revolutionized the treatment of rapidly progressing and devastating subfoveal CNV. The verteporfin-based PDT or vPDT procedure works by inducing occlusion of new vessels – this effect is achieved by light-evoked activation of verteporfin with a subsequent transfer of its energy to molecular oxygen, resulting in the formation of highly reactive singlet oxygen capable of producing direct damage of endothelial cells. Although vPDT has become increasingly prevalent, its effect on the patients' vision is limited – there is a large number of CNV recurrences after PDT and the unpredictable repetition of treatments in 3-months intervals in PDT treatment (67, 68). At present, verteporfin-based PDT alone is rather rarely used in the clinic; it can be used however in combination with anti-VEGF agent and/or steroid as a second line therapy in patients not responding to monotherapy with anti-VEGF agents (68, 69).

Anti-VEGF therapy

Currently, three anti-VEGF agents are in use in ophthalmology: bevacizumab (Avastin), ranibizumab (Lucentis) and aflibercept (Eylea). All agents are injected intravitreously under sterile conditions and require proper treatment schedule.

Avastin – bevacizumab, a full-length humanized monoclonal antibody (MW ~149 kDa) that targets all isoforms of VEGF-A family. Its basic characteristics is: bioavailability: 100%, half-life ($T_{\frac{1}{2}}$): 20 days (range: 11–50 days), dissociation constant: 0.5–1 nM, it binds with two VEGF molecules. Avastin is a drug approved in 2004 (FDA) for intravenous use in oncology. Soon, in 2005, commercially available Avastin applied intravitreously was tested in AMD patients with CNV and appeared an effective drug, becoming since then and until now the most widely used anti-VEGF agent throughout the world (due to its low cost and good efficacy comparable to Lucentis and Eylea). Avastin has no official registration for its use in wet AMD and is used on an "off-label" basis. One intravitreous injection contains 1.25 mg of bevacizumab in 50 µL of the original commercially available sterile solution (vials containing 100 mg bevacizumab in 4 mL solution).

Lucentis – ranibizumab is a recombinant humanized monoclonal antibody fragment (MW ~48 kDa) that inhibits all isoforms of VEGF-A. Basic charcteristics of ranibizumab: bioavailability: 100%, $T_{\frac{1}{2}}$ (vitreous): ~10 days, dissociation constant: 0.14 nM; it binds one VEGF molecule. In fact, ranibizumab is a modified fragment of bevacizumab showing spectrum of clinical activity similar to that

of bevacizumab. Lucentis was approved as a therapy for wet AMD in 2006 (FDA) and in 2007 (EMA). One intravitreous injection contains 0.5 mg ranibizumab in 50 µL volume.

Monthly injections of ranibizumab and bevacizumab are the current "gold standard" therapy in the management of CNV associated with AMD. The treatment schedule includes three monthly injections followed by individualized treatment regimens, including traditional PRN (pro-re-nata) and "treat-and-extend". However, as shown in preclinical studies carried out on monkeys, repeated anti-VEGF therapy strongly reduced the diameter of the choriocapillaries (70) what in consequence may lead to some undesirable effects, including atrophy of RPE-photoreceptor complex (71).

Eylea – aflibercept (also known as VEGF-Trap or VEGF-Trap-Eye) – is a soluble fusion protein (MW ~110 kDa) containing fragments of two types of VEGF receptors, VEGFR-1 (domain 2) and VEGFR-2 (domain 3), linked with the Fc fragment of human immunoglobulin G (IgG). Aflibercept behaves as a soluble decoy receptor with a dissociation constant ~0.5 pM, recognizing and neutralizing all members of VEGF-A family, VEGF-B and placenta growth factor (PlGF). It was registered for wet AMD in 2011/2012. One intravitreous dose of Eylea containins 2 mg aflibercept (50 µL), and the recommended schedule is as follows: three monthly injections followed by additional bimonthly injections (if needed); another possible variant includes bimonthly injections from the beginning of the therapy.

Macugen – pegaptanib sodium (containing polyethylene glycol – PEG). Pegaptanib is a 28-base RNA aptamer (MW ~50 kDa) that binds and neutralizes selectively only one member of VEGF-A family, i.e., VEGF-A_{165} – the most active proangiogenic VEGF-A isomer. Macugen is in fact the first drug officially registered for wet AMD (FDA, 2004; EMA, 2006). Actually, because of preferable "position" of Avastin, Lucentis and Eylea, clinical importance of Macugen substantially decreased, being in many countries not used against CNV in AMD patients. The recommended intravitreous dose of Macugen is 0.3 mg applied every 6 weeks.

Emerging therapies: ongoing or discontinued clinical trials

- Small interfering RNAs:
  siRNA-027 (bevasiranib), **PF-04523655** (REDD14). In general, siRNAs are capable of blocking protein synthesis (posttranscriptional gene silencing) for specific proteins encoded by mRNAs whose target sequences are homologues to the siRNAs.

  Bevasiranib is a double-stranded siRNA directed directly against VEGF, whereas REDD14 indirectly leads to reduction of VEGF-A production (involving a primary inhibition of hypoxia-induced gene RTP801). Bevasiranib went positively through Phases I and II of clinical trials in patients with wet AMD, but did not overcome Phase III and in March 2009 the producer announced that "OPKO Health Pharmaceuticals terminates late-stage trial of bevasiranib for treatment of wet age-related macular degeneration". Concerning REDD14, no clear-cut and definitive clinical results are available until now.
- **Squalamine lactate** (Evizon) – a naturally occurring steroidal compound conjugated to spermidine at position C-3. It prevents angiogenesis by blocking the action of VEGF and integrin expression when bound to calmodulin. In earlier clinical studies (Phase I/II) the drug's intravenous formula was used; currently, the topical squalamine lactate undergoes clinical testing in the wet form AMD.
- **Palomid 529** – an investigational medicine targeting Akt/mTOR pathway (it dissociates both targets of rapamycin complexes TORC1 and TORC2). The agent reduces tumor growth, tumor angiogenesis and vascular permeability. The drug was tested in patients with wet AMD *via* subconjunctival injections. Preliminary clinical results appeared promising, yet, no conclusive data are available.
- **KH902** – recombinant soluble VEGFR protein containing binding domains of VEGFR1 and VEGFR2 combined with the Fc portion of IgG; it shows similar properties as aflibercept.
- Tyrosine kinase inhibitors:
  Vatalanib – shows an activity against the platelet derived growth factor (PDGF) receptor and c-kit receptor kinases, and shows high oral bioavailibity. **Pazopanib** – shows slightly wider activity than vatalanib, inhibiting tyrosine kinases associated with VRGF-R, PDGF-R and c-kit. Although both drugs were clinically tested in patients with wet AMD, the results have not been published yet.

Dry – atrophic AMD

In contrast to neovascular-wet AMD, where three therapeutics are currently widely used throughout the world (Avastin, Lucentis, Eylea), there is no approved therapy for atrophic-dry AMD. Based on already accepted mechanisms underlying the dry form AMD, a number of agents were/are experimentally selected and then introduced into

clinical trials, hoping that they will be active in preventing or at least in slowing the disease course. However, many of the clinically verified compounds were withdrawn from testing for various reasons – either lack or poor clinical activity, or toxic effects, or other reasons uncovered by their producers (or distributors) – pharmaceutical company/ies. Yet, a great number of therapies proposed for atrophic AMD can be evaluated with optimism, showing continued efforts of scientists and physicians in looking for acceptable treatment of the AMD pathology. Below, some agents will be shortly presented; they include: drugs that decrease oxidative stress, visual cycle modulators, neuroprotectants, drugs that suppress inflammation, including anticomplement agents [according to: (72–75)].

- Drugs that decrease oxidative stress:
  AREDS-2 formula (Age-Related Eye Disease Study 2) – a composition of two macular xanthophylles (lutein, zeaxanthin), vitamins (E, C), copper, zinc (both in oxide form) – all ingredients possessing direct or indirect antioxidant activity A mixture contained also two ω-3 PUFAs: EPA and DHA, but their role in AMD is discussable (due to their both positive and possible negative potential (23, 61)); Phase III clinical study was completed at the end of 2013. Oral use.
  OT-551 (Tempol; a prodrug of tempol hydroxylamine – tempol-H) – SOD mimetic and NRF2 activator. Phase II. Topical application.

- Visual cycle modulators:
  ACU-4429 (Emixustat-HCl) – a small non-retinoid; it inhibits retinol isomerization by acting on RPE65 and thus slows the rod visual cycle. It is designed to prevent the generation of toxic by-products of the visual cycle. Phase II/III – the study is currently ongoing. Oral use.
  Fenretinide – a synthetic retinol analog; it inhibits retinol binding to retinol binding protein – RBP. The "positive" results of Phase II clinical trial were not statistically significant and the drug did not enter Phase III study. Oral use.

- Neuroprotectants:
  CNTF (ciliary neurotrophic factor; NT-501) – a neuroprotective cytokine that prevents photoreceptor degeneration. Phase III – ongoing. An intravitreal implant using "encapsulated cell technology".
  Brimonidine tartrate – an α_2-adrenergic receptor agonist with neuroprotective potential. It is an approval anti-glaucoma medicine (eye drops). Phase II study with AMD patients using a sustained delivery system is underway. Intravitreal implant.
  Tandospirone – a selective 5-HT_{1A} receptor agonist that as an oral version is used as anxiolytic and antidepressant; it protects photoreceptors and RPE cells from photo-oxidative stress. The drug completed Phase III trial in dry AMD in 2012, but the obtained results have not yet been released. Topical application.
  Glatiramer acetate (Copaxone) – an immunomodulatory drug currently used to treat multiple sclerosis; multifunctional agent: it suppresses T-cells, downregulates inflammatory cytokines, reduces amyloid-β-induced retinal microglial cytotoxicity. Phase II study is underway. Subcutaneous injection.

- Drugs that reduce toxic by-products:
  RN6G (PF-4382923) – a humanized monoclonal antibody against amyloid-β that accumulates in drusen. Phase II study is ongoing. Intravenous injection.
  GSK 933776 – a humanized monoclonal antibody against amyloid-β N-terminus. Phase II study is currently underway. Intravitreal injection.

- Drugs that suppress inflammation:
  ILuvien (fluocinolone acetonide) – an anti-inflammatory and immonosupressing corticosteroid. Phase II study with dry AMD patients is in progress (currently approved in Europe for diabetic macular edema). Intravitreal implant.
  POT-4 (Compstatin, AL-78898A) – a synthetic cyclic peptide that irreversibly inhibits complement C3 with resulting inhibition of complement pathways and prevention of membrane attack complex (MAC) formation. Phase II study completed, the results have not yet been released. Intravitreal injection.
  Soliris (Eculizumab) – a humanized IgG antibody against complement C5. It is the first FDA approved complement inhibitor for the treatment of paroxysmal nocturnal hemoglobinuria. It prevents lysis of erythrocytes and the formation of MAC. Phase II study with AMD patients – no clear-cut results are available. Intravenous injection.
  FCFD4514S (Anti-Factor D Fab; Lampalizumab) – a humanized monoclonal antibody (Fab fragment) against complement factor D; it inhibits complement activation and chronic inflammatory process in tissues. Phase II study

with dry AMD patients completed and Phase III trial is planned. Intravitreal injection.
LFG1905 – a fully human antibody against complement C5. Phase II study in progress.
Intravitreal injection.
ARC1905 – aptamer-based complement C5 inhibitor. Phase I clinical trial. Intravitreal injection.

- Vascular enhancers:
  MC-1101 – a facilitator of choroidal blood flow. Phase II/III study. Topical application.

- Stem cell replacement:
  MA09-hRPE – a human embryonic stem cell-derived RPE cells. Phase I/II study. Stem cell transplant.
  itMSC-DryAMD – an "ischemic tolerant" mesenchymal stem cells. Phase I/II study. Stem cell transplant.
  HuCNS-SC – a human central nervous system stem cells. Phase I/II study. Stem cell transplant.

Actually, many potential therapeutic strategies focused on complement modulation are in preclinical studies, they include (76):

- protease inhibitor (C1-INH; inhibition of C1q, C1r, C1s),
- factor B inhibitor (TA106 – Fab; inhibition of C3 convertase),
- C3-inhibitor (TT30/CR2-fH – fusion protein containing CFH and CR2; inhibition of C3 convertase),
- recombinant complement factor H (rCFH from human plasma; replacement of CFH and inhibition of C3 convertase),
- antagonist of C5aR (peptide JPE-1375/JSM-7717 and PMX53; binding C5a),
- gene therapies with: human CD46 (AdCAGCD46; inhibition of C4b and C3b) and human CD59 (AdCAGsCD59; inhibition of MAC).

Recent analysis published in the Cochrane Review series devoted to complement inhibitors for AMD provides a meaningful conclusion: "There is insufficient information at present to generate evidence-based recommendations on the potential safety and efficacy of complement inhibitors for prevention or treatment of AMD. However, we anticipate the results of ongoing trials." (77).

Comment on dietary supplement-based treatment for dry AMD

One of the mentioned therapies, i.e., AREDS-2 study/formula, needs comment, because it deals with an "antioxidant" dietary supplementation that in fact was and still is widely used by AMD patients, as well as by people being at risk of developing the disease. The concept that antioxidant agents may prevent the development and/or the course of AMD finds its rationale in the disease pathogenesis, where oxidative stress seems to be one of driving forces (see Figs. 2 and 3). Various antioxidant preparations were in the past and still are freely available on the market – such mixtures were recommended by ophthalmologists to their patients, becoming in fact the only modality with which to prevent or "cure" atrophic-dry form AMD. A "scientific" support for beneficial role of antioxidants in AMD came from AREDS (or AREDS-1) clinical trial whose primary aim was to evaluate the effect of pharmacological doses of nutritional supplements on the rate of progression to advanced AMD. The AREDS formula included: vitamin C (500 mg), vitamin E (α-tocopherol; 400 IU), β-caroten (15 mg), zinc (as zinc oxide; 25 or 80 mg) and copper (as cupric oxide; 2 mg) – the results were published late in 2001. According to the 2001 report, AREDS formula led to an overall 19–25% risk reduction in the disease progression in individuals who had a moderate risk of AMD development at five years' treatment (78). At that time, many enthusiastic opinions on clinical efficacy of AREDS formula announced on different occasions have appeared, including NEI Information Office – News and Events (October 2001): "Antioxidant vitamins and zinc reduce risk of vision loss from age-related macular degeneration". However, since then, a number of less enthusiastic opinions has also been published (13, 14). Interestingly, somewhat earlier it has been observed in ATBC (α-tocopherol, β-carotene cancer prevention) study that β-carotene contributes to the development of lung cancer in smokers (79). In addition, β-carotene is a precursor of vitamin A, and the visual cycle retinoids (vitamin A derivatives) may act as substrates for the formation of photocytotoxic bisretinoids (e.g., A2E). Beneficial effects of zinc have been known for a long time, however, a question arose with regard to the dose: 25 or 80 mg – which should be used? And, finally, recently there was a trend to supplement AMD patients with lutein and zeaxanthin – two naturally occurring macular pigments (xanthophylls), as well as ω-3 PUFAs (EPA, DHA), with a practical, although unproven idea: the more agents the better. Having this in mind, AREDS-2 large research project – clinical multicenter randomized study involving more than 4,000 participants (sponsored by National Eye Institute – NEI with collaboration from National Heart, Lung,

and Blood Institute – NHLBI) was initiated in 2007 and lasted till the end of 2012; its aim was to evaluate the effect of oral supplementation with macular xanthophylls (lutein, zeaxanthin) and ω-3 PUFAs (DHA, EPA) on AMD progression. The AREDS-2 formula consisted of: lutein (10 mg), zeaxanthin (2 mg), vitamin C (500 mg), vitamin E (400 IU), copper (as cupric oxide – 2 mg), EPA (650 mg), DHA (350 mg). Smaller studies (Secondary Randomization Agents – AREDS-Type Supplement) were also conducted to examine the effects of zinc (as zinc oxide) at doses of 25 and 80 mg, with particular focus on the lower dose, and elimination of β-carotene from the AREDS formula. The results of the AREDS-2 study were published in May 2013 (15) with general conclusion as follows: "Addition of lutein + zeaxanthin, DHA + EPA, or both, to the AREDS formulation in primary analysis did not further reduce risk of progression to advanced AMD. However, because of potential increased incidence of lung cancer in former smokers, lutein + zeaxanthin could be an appropriate carotenoid substitute in the AREDS formulation." In other words, there was some benefit to patients taking macular xanthophylls (instead of β-carotene), when the patients' diet was deficient in these pigments, ω-3 PUFAs did not improve the original AREDS formula, there was no difference between low dose and high dose of zinc.

In conclusion, there are still several unanswered questions concerning the described dietary supplementation, including, for example, the length of treatment necessary to see any improvement in patients' vision (whatever this means). And finally, based on recently published vast literature on diet and dietary supplements in AMD, as well as unpublished various (in terms of therapeutic output) observations of numerous ophthalmologists, one can say that pharmaceutical supplementation may, but does not have to help the AMD patients, leaving an open question on its effectiveness in practice (13). However, one should not cease being optimistic; optimism should be cherished by both the physicians when recommending appropriate dietary supplements to patients, and patients who would be regularly taking it; otherwise, the supplementation would serve no purpose whatsoever.

Yet, one should believe that the efforts of scientists – chemists, pharmacologists, physicians-ophthalmologists – will finally culminate in finding effective therapy for AMD, particularly for its widely occurring atrophic-dry form, that will find general acceptance as a therapeutic "gold standard".

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