

Review Article

Current concept of the pathogenesis of age-related macular degeneration: the role of oxidative stress and inflammation

Yutaka Imamura*, Susumu Ishida, Kazuo Tsubota

Department of Ophthalmology, Keio University School of Medicine

Age-related macular degeneration (AMD) is a common disorder in the aged people that affects the central region of the retina, which is called macula. AMD is the leading cause of legal blindness in people older than 65 in the developed world. AMD is characterized by very typical retinal pathologies including drusen, retinal pigment epithelium dysfunction, retinal degeneration (geographic atrophy) and choroidal neovascularization. Recent studies reveal that oxidative stress and inflammation are significantly linked to the pathogenesis of AMD. Retina is especially susceptible to oxidative stress by following reasons: 1) photoreceptors contain polyunsaturated fatty acids, 2) retina is exposed continuously to light, 3) its oxygen consumption is high, 4) it contains chromophores which react with light and produce reactive oxygen species (ROS). Moreover, randomized, prospective, placebo-controlled study in United States showed that high-dose supplementation of beta-caroten, vitamin C, vitamin E, copper, and zinc significantly slowed the progression of the disease and visual deterioration in patients with AMD. Mice deficient of Cu, Zn-superoxide dismutase showed typical features of human AMD, suggesting that oxidative stress scavenger may play a critical role in the prevention of age-related retinal degeneration. Obesity and inflammatory biomarkers are significantly associated with the risk of AMD, which possibly indicates that AMD is considered as a consequence of disturbance of homeostasis, such as metabolic syndrome. Recent genetic studies showed genes related to immune system via complement are associated with AMD, which revealed that inflammatory reactions are causally linked with the pathogenesis of AMD. Recently, the inhibitor of angiotensin II type 1 receptor reduced the volume of experimental CNV after laser burn, suggesting that angiotensin II type 1 receptor blockade be a novel therapeutic strategy as a preventive treatment for AMD. Further research is necessary to invent mechanism-based therapies for AMD.

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*Correspondence should be addressed to:

Yutaka Imamura, MD., Department of Ophthalmology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. e-mail:imamura@sc.itc.keio.ac.jp

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Introduction

Age-related macular degeneration (AMD) is a common disorder in the elderly that affects the central region of the retina, which is called macula¹⁾. AMD is the leading cause of legal blindness in people older than 65 in the developed world²⁻⁶⁾. The prevalence of AMD will rise as the population ages. AMD is a clinically heterogeneous and complex disorder¹⁾. Population-based longitudinal studies have established that the presence of extracellular protein between the basal lamina of the retinal pigment epithelium (RPE) and the Bruch's membrane is associated with an increased risk of progressing to an advanced form of AMD, either geographic atrophy or choroidal neovascularization¹⁾. The presence of large and soft drusen and RPE abnormalities is considered to be an early form of the disorder. Since AMD causes significant visual disturbances in the elderly, it is important to understand its pathogenesis, which then leads to the identification of proper preventive modalities. Recently, cumulative evidences suggest the role of oxidative stress and inflammatory reactions in the pathogenesis. Here, we review the current concept and evidence which indicate the association of oxidative stress and inflammation with AMD.

Reactive Oxygen Species in Retina: basic physiology

Retina is considered susceptible to oxidative stress because 1) photoreceptors contain polyunsaturated fatty acids, 2) retina is exposed continuously to light, 3) its oxygen consumption is high, 4) it contains chromophores which react with light and produce reactive oxygen species (ROS)⁷⁾.

Recently our group reported that mice deficient of Cu, Zn-superoxide dismutase (SOD1) showed typical features of AMD including drusen, choroidal neovascularization, RPE dysfunction⁸⁾. This results showed for the first time the genetic evidence that oxidative stress plays a causative role in AMD. Moreover, SOD1-deficient mice appear to recapitulate well features of human

AMD, indicating that they will be valuable animal models to study human AMD. We summarized the characteristics of representative animal models of AMD reported so far (Table 1).

1) Polyunsaturated fatty acids in retina

The photoreceptor membranes of both rods and cones contain a lipid bilayer which provides a matrix that is passively permeable to ions, thereby accommodating subcellular compartmentalization and the stabilization of membrane proteins such as rhodopsin⁷⁾. Polyunsaturated fatty acids (PUFA) account for about 50% of the lipid bilayer of rod outer segment membranes, and proteins make up the remaining 50%. Docosahexanoic acid (DHA) makes up about 50% of the rod photoreceptor phospholipids. This high proportion of long-chain PUFAs found among all phospholipid classes appears very unique in retina. DHA and its precursor, the essential fatty acid α -linoleic acid, are entirely of dietary origin. As DHA contains six double bonds, and as the susceptibility of unsaturated fatty acids to oxidation correlates directly with the number of double bonds, the retina is inherently susceptible to lipid peroxidation⁷⁾. Lipid peroxidation of membrane PUFAs results in loss of membrane function and structural integrity. Another recent study have examined possible associations between dietary intake and progression of AMD⁹⁾, based on a proposed atherosclerotic pathogenesis of AMD¹⁰⁾.

2) Retinal irradiation

Ultraviolet and visible radiation has the potential to damage the retina and pigment epithelium. The human retina is protected from short-wavelength radiation, which is particularly damaging, by the cornea which absorbs below 295 nm and the lens which absorbs strongly below 400 nm¹¹⁾. Retinal injury following irradiation was demonstrated by the study the histopathologic findings of 20 rhesus monkey retinas that had been exposed to blue light (441 nm) for 1000 seconds¹²⁾. The main effect of light exposure appears selective degeneration of photoreceptors, a reduction of the long-chain polyunsaturated fatty acid. Both the loss of PUFAs and the increase in conjugated dienes,

Table 1 Characteristics of animal models of AMD previously reported

genes deficient in mice	CNV(LM)	CNV(EM)	drusen(LM)	retinal degeneration	Ig deposit	ref
<i>Ccl2</i> ^{-/-} or <i>Ccr2</i> ^{-/-}	No	Yes	No	Yes	Yes	8
<i>Sod1</i> ^{-/-}	Yes	Yes	Yes	Yes	Yes	41
<i>Cp</i> ^{-/-} and <i>Heph</i> ^{-/-}	No	Yes	No	Yes	No	42

LM: light microscopy, EM: electron microscopy, Ig: immunoglobulin

an accepted measure of lipid hydroperoxides, provide compelling evidence that lipid peroxidation plays a role in retinal light damage. It was noted that short-wavelength light resulted in damage to the photoreceptor outer segments, cellular proliferation, and mitotic figures in the RPE and choroid, and hypopigmentation of the RPE, which resembled atrophic changes seen in AMD. It was found that the power required to cause photic damage was 70 to 1000 times lower for blue light (441.6 nm) than for the infrared wavelengths (1064 nm)¹¹.

3) Oxygen consumption in retina

The retina is an ideal environment for the generation of reactive oxygen species for several reasons. Oxygen consumption by the retina for the oxidative metabolism was described as higher than that of the brain on a per-gram basis, thereby suggesting that the oxygen consumption by the retina is much greater than by any other tissue¹³. Oxidation refers to the removal of electrons and reduction refers to the gain of electrons. Energy from dietary carbohydrates, proteins, and lipids by oxidizing them to CO₂ and H₂O. A series of reactions known as the TCA cycle is responsible for most of the oxidation of fuels. The energy is then conserved in the form of the reduced electron-accepting coenzymes. The electrons of these coenzymes can be used to reduce oxygen to H₂O via the electron transport chain, and this reaction releases energy and creates adenosine triphosphate (ATP). Oxidative phosphorylation occurs in the mitochondrion and is catalyzed by ATP synthase. The electron transport chain accounts for approximately 90% of our total O₂ consumption. ROS continually leaks from the active sites of the enzymes involved in oxidative processes. Other factors known to increase the production of ROS include irradiation, aging, inflammation, raised partial pressure of O₂, air pollutants, cigarette smoking, and tissue injury following reperfusion.

In vascularized retina, the highest oxygen tension remains at the Bruch's membrane close to the choroidal vasculature¹³. The oxygen tension slowly increases in the superficial retina toward the inner limiting membrane. Since AMD is considered to be caused by RPE dysfunction, high oxygen pressure surrounding Bruch's membrane may explain the susceptibility of oxidative stress to RPE by oxygen consumption.

4) Retinal chromophores

Chromophores, or photosensitizers, are molecules that absorb light to produce a chemical reaction that would not occur in their absence. Photochemical damage may be defined as injury arising from absorption of UV and visible light by a chromophore, which results in an electronic transition of the substrate to the excited state⁷. The retinal chromophores include rhodopsin,

melanin, lipofuscin, and the mitochondrial respiratory enzymes, such as cytochrome c oxidase. There are three types of retinal photopigments: 1) cone photoreceptor photopigments that provide photopic (bright light) and mesopic (intermediate light) vision, 2) rhodopsin in rod photoreceptors responsible for scotopic vision, and 3) melanopsin in blue light sensitive retinal ganglion cells that modulate circadian photoentrainment and pupillary function¹⁴. However recent study suggests that melanin has a protective effect to oxidative damage. Decrease of melanin concentration in aging may contribute to the pathogenesis of AMD¹⁵.

Evidence collected since 1970s suggests that light may damage the retina in a number of ways involving different chromophores. Longer exposures to much less-intense light sources may cause retinal damage by a photochemical mechanism. Retina is highly organized tissue capable of light stimulation to sensation, however on the contrast it continuously being suffered from photo-oxidative damages.

Age-related Eye Disease Study: a clinical evidence of the antioxidant therapy for AMD¹⁶⁻²⁰

Oxidative damage to the retina may be involved in the pathogenesis of AMD. Therefore, it has been long considered that supplementation of antioxidant can reduce the risk of AMD. However, data from epidemiological studies as well as small randomized clinical trials do not show consistent associations between intake of antioxidants or zinc and risk of AMD. One small, randomized, 2-year, placebo-controlled clinical trial of zinc supplementation found a statistically significant reduction in visual acuity loss in the zinc-treated group and recommended a more definitive trial before a general recommendation could be made for zinc supplementation in those at risk of vision loss from advanced AMD²¹. Despite the lack of convincing evidence, the marketing and use of antioxidants and zinc in eye-targeted formulations has become a common practice. Therefore, high-dose antioxidant and zinc supplements for AMD led the National Eye Institute (National Institutes of Health, Bethesda, Md) to incorporate a clinical trial as part of the Age-Related Eye Disease Study (AREDS).

AREDS reported the treatment with zinc and antioxidants reduced the risk of progression to advanced AMD and vision loss in patients who have high-risk characteristics lesions defined by extensive intermediate drusen, large drusen, noncentral geographical atrophy in one or both eyes (category 3) or if they have advanced AMD (GA involving centre of the macula or signs of choroidal neovascularization: category 4) in the first eye. AREDS

did not show benefit of supplementary nutrients in patients with milder drusen and RPE abnormalities. (category 1 and 2).

Although both zinc and antioxidants plus zinc significantly reduce the odds of developing advanced AMD for participants in Categories 3 and 4, the only statistically significant reduction in rates of at least moderate visual acuity loss occurred in persons taking antioxidants plus zinc. Persons with history of past or current smoking should avoid taking beta-carotene, because it was reported that it increased the risk of lung cancer in people with history of smoking. Based on data from AREDS, it is highly recommended that persons older than 55 years should have dilated eye examinations to determine their risk of developing advanced AMD. Those with extensive intermediate size drusen, at least 1 large druse, or noncentral GA in 1 or both eyes or those with advanced AMD or vision loss due to AMD in 1 eye, and without contraindications such as smoking, should consider taking a supplement of antioxidants plus zinc such as that used in this study.

AREDS revealed the efficacy of antioxidant therapy for AMD, and moreover this study strongly suggested that oxidative stress may causatively associated with AMD in humans.

Obesity and AMD²²⁾

Body weight is a strong predictor of coronary heart disease risk and death from CVD. Since AMD is significantly associated with the occurrence of cardiovascular diseases, obesity may be a possible risk factor of AMD. In a study in Australia, a J- or U-shaped association was observed between BMI and early AMD, diagnosed by fundus photographs²³⁾. Similarly, in the different study, the relationship of BMI with advanced AMD appeared to be J-shaped, with the highest incidence among obese men with a BMI of at least 30 and a somewhat less elevated incidence among the leanest men with a BMI less than 22. These results appear to show AMD as a consequence of irregular homeostasis. One study could identify no significant relationship of BMI with neovascular AMD, but the number of participants with this late form of macular degeneration was relatively small. Further study of whether BMI is a risk factor for neovascular AMD in studies of sufficient size is needed. Recently, common systemic diseases such as hypertension, diabetes, cardiovascular diseases, are considered as a consequence of common metabolic disorder, called metabolic syndrome. Since AMD patients have common systemic diseases than subjects without AMD, AMD can be considered as a consequence of metabolic syndrome.

AMD is significantly associated inflammatory markers

Recent clinical study found that both CRP and IL-6, markers of systemic inflammation, were significantly and independently related to AMD after adjustment for known and potential confounding factors²⁴⁾. When subjects divided in 4 groups, the highest quartile of CRP was significantly associated with progression of AMD, with a twofold greater risk compared with the lowest quartile of CRP. The highest quartile of IL-6 was significantly related to progression of AMD, with twofold greater risk compared with the lowest quartile. For CRP, the estimates of risk were increased above the first quartile. The IL-6 values in the third and fourth quartiles were associated with increased risk, and the trend for increasing risk of AMD with increasing levels of IL-6 was statistically significant. These results strengthen and expand the previously reported finding of association between CRP and advanced AMD^{25,26)}. The study group concluded that anti-inflammatory agents may have a role in preventing AMD, and inflammatory biomarkers may provide a method of identifying people for whom these agents would be more or less effective, which may be valuable to calculate risk of progression of AMD in each patient.

Complement factor H and AMD: genetic evidence of the role of inflammation in the pathogenesis of AMD²⁷⁻³⁰⁾

Complement factor H (CFH) is a component of the immune system which helps to regulate the body's inflammatory response by protecting against uncontrolled complement activation. The Tyr402His variant is located within binding sites for heparin and C-reactive protein. Binding to either of these factors increases the affinity of CFH for complement protein C3b, enhancing CFH's ability to inhibit complement's effect. Recently, three separate research groups identified a common coding variant in CFH which appears to exert a strong influence on the risk of developing AMD. This variant, Tyr402His, was associated with odds ratios ranging from 2.45 to 3.33 for all stages of AMD. Odds ratios ranged from 3.45 to 7.4 for the more advanced stages of AMD including geographic atrophy and neovascular disease. The results of these investigations suggested that close to one half of all AMD cases in older adults may be attributable to the CFH gene. Since the time of these publications, six additional reports have confirmed the role of CFH in AMD development. Although all studies have pointed to the Tyr402His variant, other significantly associated variants within CFH have also been identified, including several protective haplotypes. The other major loci for

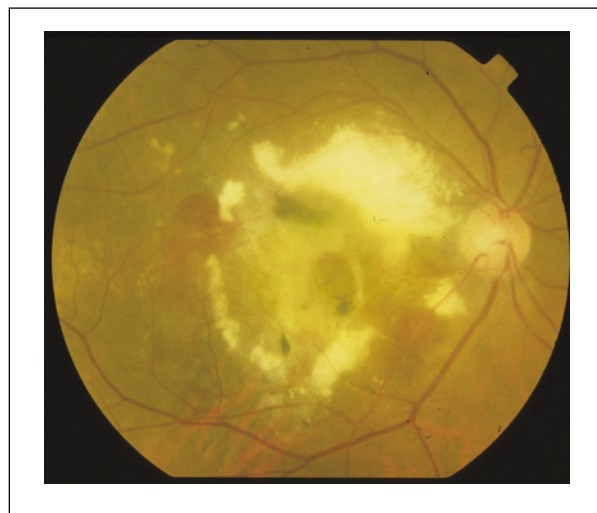


Fig.1 Fundus photograph of adavanced AMD
Exudative change and subretinal fibrosis due to choroidal neovascularization is observed.
At this stage, no effective treatment is available.

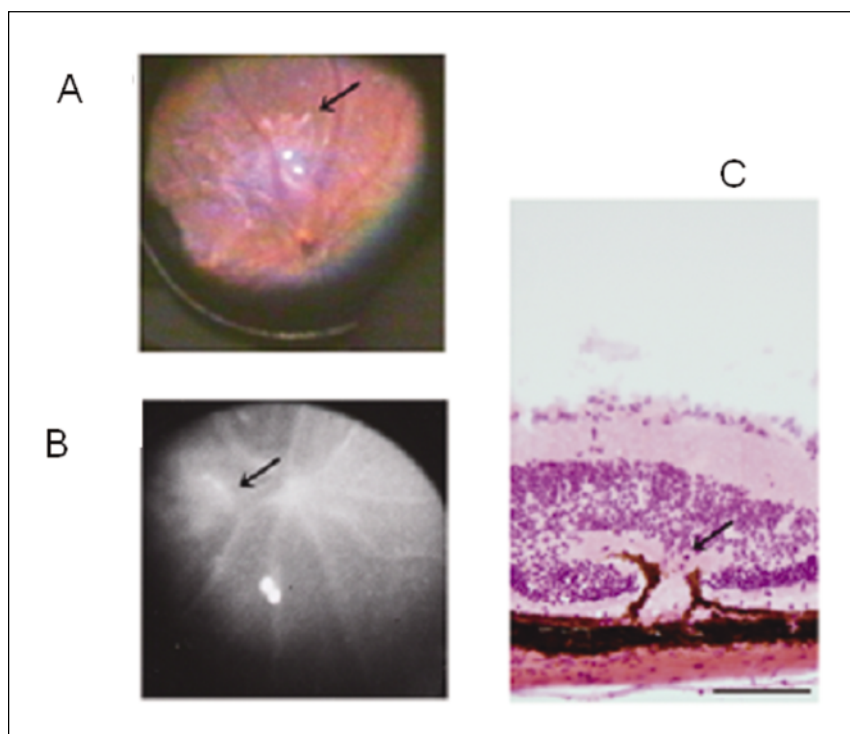


Fig.2 SOD1-deficient mouse showing choroidal neovascularization
(ref.3, reprint permitted from PNAS)

AMD have been identified. These genes include LOC387715³¹⁾, BF, and C2³²⁾. Although the function of LOC387715 is unknown, inflammatory reaction via complement system appear play a critical role in the pathophysiology in the AMD. Several other studies suggest other possible linkages of AMD³³⁻³⁸⁾.

Renin-angiotensin system in the development of the experimental CNV: the possibility of AT-R blockade as a novel therapeutic strategy³⁹⁾

Epidemiologic risk factors for AMD include hypertension,

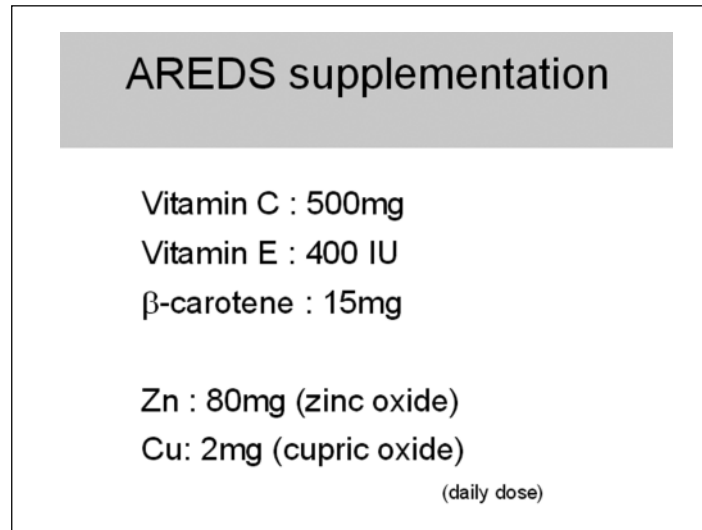


Fig.3 AREDS supplementation

High dose intake of antioxidant significantly reduced the progression of AMD.

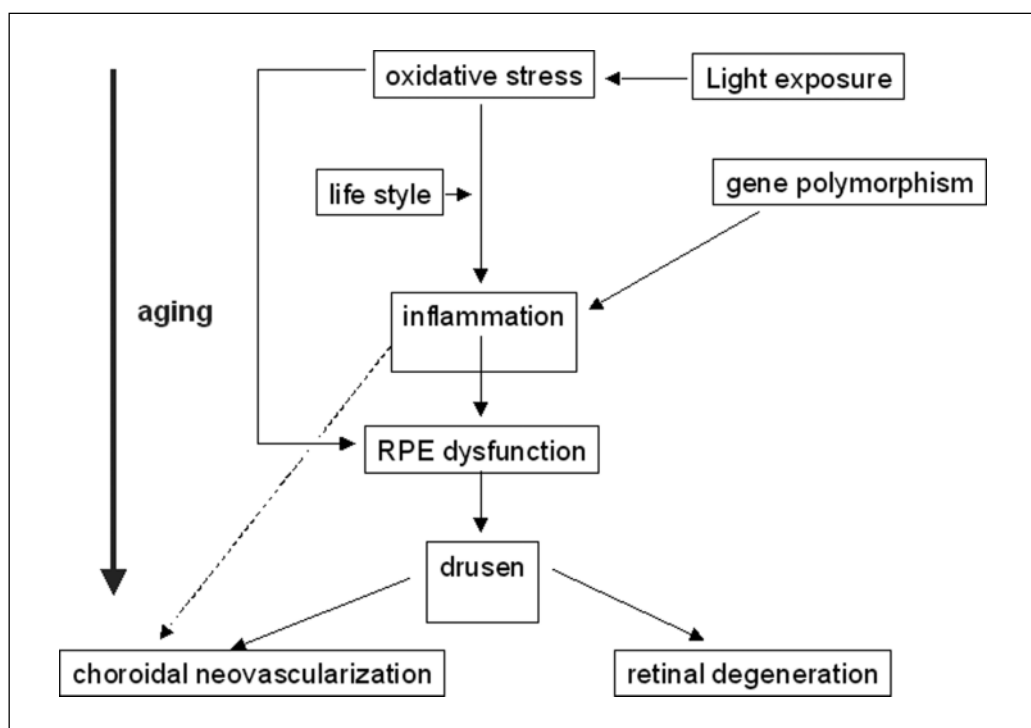


Fig.4 Diagram showing the pathogenesis of AMD

atherosclerosis, and hyperlipidemia, all of which are the main components of the metabolic syndrome. Recently our group reported that the inhibitor of angiotensin II type 1 receptor significantly reduced the volume of experimental CNV after laser burn. AT1-R signaling blockade with telmisartan inhibited various

inflammatory reactions including macrophage infiltration and upregulation of VEGF, intercellular adhesion molecule-1 (ICAM-1), MCP-1, and IL-6³⁹). A PPAR- γ antagonist partially but significantly reversed the suppressive effect of telmisartan on *in vivo* induction of CNV and *in vitro* upregulation of ICAM-1 and

MCP-1 in endothelial cells and IL-6 in macrophages. These results showed the dual contribution of PPAR- γ agonistic and AT1-R-antagonistic actions in the telmisartan treatment³⁹. Currently, the treatments for AMD complicated by CNV include photodynamic therapy⁴⁰ and anti-VEGF therapy⁴¹. Because these therapeutic interventions target the advanced stage when the visual function is irreversibly impaired, alternative early treatment is required. AT1-R blockade, which not only inhibits inflammatory neovascularization in the eye but also improves the systemic background such as hypertension, is likely to be a novel therapeutic strategy as a preventive treatment for AMD.

Conclusion

The past decade has showed remarkable advance in the study of AMD, including clinical study, the development of diagnostic modalities, isolation of many genes responsible for AMD. The oxidative stress and subsequent inflammatory reaction may play a causative role in the pathogenesis of AMD. The trend of current research strongly implies that AMD has impairment of homeostasis in its background, may be considered as a consequence of metabolic syndrome. Preventive modalities including wearing sunglass, antioxidant supplementation, dietary intake of fish and carotenoid, may appear particularly useful to stop progression of AMD, although appropriate randomized clinical trial is necessary to establish the efficacy of each modality. Further research would be necessary to invent effective mechanism-based therapy of this progressive dysfunction of retina, leading to visual disability among adults in industrialized societies.

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